



# European conference on *Xylella* 2017

Finding answers to a global problem

Palma de Mallorca, 13-15 November 2017

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## Conference

A major scientific conference on European research into *Xylella fastidiosa* (*X. fastidiosa*) is held in Palma de Mallorca, Spain, from 13-15 November 2017. The conference is being organised jointly by EFSA, the University of the Balearic Islands, the Euphresco network for phytosanitary research coordination and funding, the EU Horizon 2020 projects POnTE and XF-ACTORS, and the European Commission's Directorate-General for Research and Innovation (DG RTD).

The event provides a platform for in-depth discussion on the results of research into *X. fastidiosa* and its vectors, in support of on-going efforts to control the European outbreaks. As well as speakers and participants from Europe, the conference will be attended by scientific experts from other parts of the world where *X. fastidiosa* has been present for many years.

The recent detection of *X. fastidiosa* in Italy, France and Spain represents an important change in the geographical distribution of this dangerous plant pathogen, which now poses a serious threat to the agriculture and environment of Europe and the Mediterranean region.

This year's conference follows the workshop *X. fastidiosa*: knowledge gaps and research priorities for the EU that was organised in 2015 by EFSA and the European Commission's Directorates-General for Research and Innovation, Agriculture and Rural Development, and Health and Food Safety<sup>1</sup>.

This scientific conference is dedicated to the presentation and discussion of results of European research projects on *X. fastidiosa* and its vectors. Priority for participation will be given to scientists already involved in research on *X. fastidiosa*, or who are embarking on new research into the topic. Risk assessors, risk managers and stakeholders will also be important contributors to the discussion.

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<sup>1</sup> <https://www.efsa.europa.eu/en/events/event/171113>

## Conference Programme

### 13 November 2017

- 8:30 – 8:45 Welcome and introduction to the event
- 08:45 - 10:50 Session 1 | *Xylella fastidiosa*: a global threat
- 11:20 - 13:05 Session 2 | *Xylella fastidiosa*: biology and genetics
- 14:05 - 16:10 Session 3 | *Xylella fastidiosa*: host plants and resistance
- 16:40 - 18:50 Session 4 | *Xylella fastidiosa*: pathogen and disease control in the host plants
- 18:45 - 21:00 Poster Session and Networking reception
- 20:00 – 21:00 Special parallel session CURE-XF

### 14 November 2017

- 8:30 - 12:40 Session 5 | Vectors and epidemiology
- 13:30 – 15:30 Session 6 | Detection and identification
- 16:00 – 18:00 Session 7 | From field detection to disease dynamics
- 18:00 - 20:30 Poster session and Networking reception

### 15 November 2017

- 8:30 – 10:30 Session 8 | Surveillance and control: from modelling to stakeholders analyses
- 11:00 – 13:00 Final Session

# Oral Presentations

## Session 1 - *Xylella fastidiosa*: a global threat.

### *Xylella fastidiosa*, new risks from an old threat

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**Abstract:** *X. fastidiosa* has long been considered a major threat to European flora. However limited interest had been paid to this exotic bacterium, as it had never been shown to be established in Europe. This changed significantly and rapidly after the detection of *X. fastidiosa* in southern Italy in 2013, followed by a series of reports primarily from France and Spain. In the span of just four years the status of *X. fastidiosa* at several Mediterranean regions went from absent to established. In addition, *X. fastidiosa* was introduced multiple independent times, and much of the known diversity of this pathogen is represented in these introductions. In this context, some of the emerging risks to European flora will be discussed.

### Biology and pathogenicity of *Xylella fastidiosa* associated to olive quick decline syndrome

Saponari M.\*, Boscia D., Altamura G., Loconsole G., Zicca S., D'Attoma G., Morelli M., Palmisano F., Saponari A., Dongiovanni E., Cavalieri V., Savino V.N., Martelli G.P.

\*CNR, Istituto per la Protezione Sostenibile delle Piante, Bari (IT)

**Abstract:** A large program of mechanical inoculations and vector-mediated transmission experiments was launched in the past 2 years, and continuously implemented, in the attempt to disclose the role of the olive-infecting strain of *X. fastidiosa* in the olive quick decline disease (OQDS) and to ascertain the crop species under threat due to the expansion of the severe epidemic in southern Italy. Artificial inoculations were performed using the selected olive strain "De Donno" on different olive and grape cvs, stone fruit and citrus species, and ornamentals. These plant species, but including a larger number of olive cultivars, were also tested through field experiments set in the demarcated infected area, by exposing *Xylella*-free plants to the natural inoculum pressure and/or by caging naturally infected *Philaenus spumarius*. Comparison of the bacterial infection rates recovered upon mechanical inoculations and vector transmission (field experiments), showed perfect agreement regarding the susceptibility of the different host species: (i) high rates of systemically infected plants were obtained for olives, with some differences in relation to the cultivars, oleanders and *Poligala myrtifolia*; (ii) very low host colonization occurred for cherry and almond plants; (iii) no bacterial movement and host colonization could be detected in grapes, citrus, apricot, peach and plum. The pathogenicity of this strain has been also demonstrated; severe symptoms were detected on the most susceptible hosts.

#### Acknowledgment

This work was supported by the European Food Safety Authority (EFSA) in the framework of the "Pilot project on *X. fastidiosa* to reduce risk assessment uncertainties" (NP.EFSA.ALPHA.2014.07) and by the European Union's Horizon 2020 Research and Innovation Programme, under grant agreement No. 635646, POnTE (Pest Organisms Threatening Europe).

### Emergence of *Xylella fastidiosa* in Balearic Islands, Spain: Current situation

Olmo D., Montes-Borrego M., Nieto A., Adrover F., Urbano A., Beidas O., Juan A., Marco E., López M.M., Moran F., Monterde A., Navas-Cortés J.A., Landa B.B.\*

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**Abstract:** During official surveys in late autumn 2016 in Mallorca Island, Spain, the bacterium was first detected in a garden center near the locality of Manacor. Since then, as of April 24, 2017, a total

of 189 positive samples in 11 different host species have been found in different foci in Mallorca (124), Menorca (16) and Ibiza (49) islands. Sequence analysis of the RNA polymerase sigma 70 factor sequence and MLST typing revealed the presence of *X. fastidiosa* subsp. *fastidiosa* ST1 and *X. fastidiosa* subsp. *multiplex* ST6\* (a new ST with the closest being ST6) and ST7 in Mallorca island, *X. fastidiosa* subsp. *multiplex* ST6\* in Menorca island and *X. fastidiosa* subsp. *pauca* ST80 (a new ST) in Ibiza island. *Polygala myrtifolia* was found to be infected by all subspecies and ST types. Altogether, these results suggest that the emergence of *X. fastidiosa* in Balearic Islands is likely due to several introduction events of diverse strains from different subspecies. Eradication measures were taken in the garden center according to the Spanish contingency plan and EU legislation. Following the Commission Decision 2015/789/EU of establishing a radius of 10 km to delimit the buffer zone for each infected foci 80%, 50% and 90% of the territory of Mallorca, Menorca and Ibiza islands, respectively, are considered as demarcated areas. Consequently, the best strategy to control the different outbreaks is under study.

#### Acknowledgment

This work has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N. 635646 "Pest Organisms Threatening Europe POnTE" and grant agreement N. 727987 "*Xylella fastidiosa* Active Containment Through a multidisciplinary-Oriented Research Strategy XF-ACTORS".

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### ***Xylella fastidiosa* in France: current situation in Corsica and in the region of Provence-Alpes-Côte d'Azur.**

Poliakoff F.\*, Legendre B., Olivier V.; Dousset C., Paillard S., Molusson D., Sainte- Luce A., Juteau V., Cuntz A.

\*ANSES, Angers (FR)

**Abstract:** Since the first outbreak of *X. fastidiosa* on *Polygala myrtifolia* (Pm) in natural settings in 2015 in France, the national survey showed that disease is present in many ornamental host plant in environment of Corsica and French Riviera (PACA). *X. fastidiosa* has been detected on around forty plant species with a validated method based on Real-Time PCR (Harper *et al.*, 2010) associated to DNA extraction with QuickPick™ Plant DNA kit (Bio-Nobile) and KingFisher™ automate (Thermo Fisher Scientific). The sample preparation and isolation performed on modified PWG medium (EPPO, 2016) have been optimized and more than 40 *X. fastidiosa* strains were isolated from various ornamentals and trees. The characterization of isolates directly on plant or on pure strains is performed according to a multilocus sequence typing (MLST) ([http://pubmlst.org/X. fastidiosa/](http://pubmlst.org/X.fastidiosa/)). Following EPPO protocol PM 7/24 isolates were mostly allocated to sequence types ST6 and ST7 (subspecies *multiplex*). Modifications of the amplification protocol (Yuan *et al.*, 2010) proposed by Denancé *et al.* (2017) revealed infections linked to the subspecies *pauca*, *sandyi*, one recombinant and some mixed infections. EPPO protocol MLST confirmed four isolates in *Polygala myrtifolia* from PACA contaminated with subsp. *pauca* but not the identification of other contaminants. These contaminations could not be observed again in the immediate environment after plant eradication. Subspecies assignment directly from plant material is not always successful linked to PCR inhibitors depending of host plants. This study confirms the diversity of subspecies of *X. fastidiosa* in France. Nevertheless subspecies *multiplex* was found in the great majority.

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## ***Xylella fastidiosa* in Costa Rica: understanding of the pathogen and containment through collaborative approach**

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**Abstract:** *X. fastidiosa* is endemic in Costa Rica. Since the 1990's, the appearance of a milder version of CLS was reported as "crespera" in Costa Rica. It was confirmed that the disease was due to *X. fastidiosa* infection. Since then, the bacterium has been detected and isolated from more than 20 different economic important crops and ornamentals. Although the bacterium has great potential for disease and it is widespread throughout the country, the symptoms related to infected plants are usually mild or asymptomatic. In 2015, the European Union closed the importation of ornamentals from Costa Rica, alleging that *X. fastidiosa* was introduced in this matter into Europe, causing great social and economic impact in this activity in Costa Rica. Recently, genomic evidence supports the relatedness of the CoDiRo strains to *X. fastidiosa* ST53 isolates from Costa Rica. Our participation as part of the PonTE and XFactors research efforts are focused on broadening the genetic and phenotypic information related to our circulating strains, this will contribute to elucidate some specific traits of the *X. fastidiosa* strains found in Italy as in other European countries as well. We are also working on alternative containment strategies for the bacteria. Finally, we have established research initiatives related to endusers in collaboration with phytosanitary authorities to determine the host susceptibility to *X. fastidiosa* of economic important plants such as *Phoenix roebelenii*.

### *Acknowledgment*

This work has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N. 635646 "Pest Organisms Threatening Europe POnTE" and grant agreement N. 727987 "*Xylella fastidiosa* Active Containment Through a multidisciplinary-Oriented Research Strategy XF-ACTORS".

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## **Session 2 - *Xylella fastidiosa*: biology and genetics**

### **Calcium has multiple roles during the interactions between *Xylella fastidiosa* and host plants**

De La Fuente L.\*, Hongyu C., Sy T., Parker J. K., Cruz L. F., Oliver J. E. and Cobine P. A.

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**Abstract:** Inside the plant host *X. fastidiosa* is restricted to live inside xylem vessels, the vascular system where mineral elements are transported from the roots to the rest of the plant. Previous research by our group has demonstrated that calcium (Ca) increases movement and biofilm formation of *X. fastidiosa* by manipulating protein secondary structure and regulating gene expression. Interestingly, plants infected with *X. fastidiosa* also show accumulation of Ca in their leaves and xylem

sap. Further studies on bacterial proteins involved in the response to Ca identified mopB, an outer membrane protein that is important for biofilm formation and fundamental for twitching motility. Deletion of this protein in two wild-type strains caused severe reduction of virulence. Regulation of gene expression by Ca was also studied under flow conditions inside microfluidic chambers by whole transcriptome analysis (RNA-Seq), revealing novel gene targets not identified in our previous batch culture studies. From the plant host perspective, we show that addition of Ca by watering causes an increase in *X. fastidiosa* caused symptoms in tobacco plants. A study of the genes involved in the plant response to Ca lead us to specific Ca transporter that are upregulated during infection. *X. fastidiosa* has evolved to live in an environment such as the xylem sap that is rich in Ca, and our studies revealed the importance of Ca as a modulator of virulence of *X. fastidiosa* in a phenomenon similar to nutritional immunity in animal system.

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- Oliver, J.E. *et al.* Ionome changes in *Xylella fastidiosa*-infected *Nicotiana tabacum* correlate with virulence and discriminate between subspecies of bacterial isolates. *Molecular Plant-Microbe Interactions* 27, 1048-1058 (2014)

### **XfDSF II are found in *Xylella fastidiosa* outer membrane vesicles**

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**Abstract:** *X. fastidiosa* produces a family of Diffusible Signal Factors (DSF), which are 12-18 carbon unsaturated fatty acids released to the extracellular milieu (Beaulieu *et al.*, 2013). DSF are known to act through the RpfC signaling pathway to modulate expression of virulence factors such as adhesion proteins, exopolysaccharide, plant cell wall degrading enzymes as well as production of outer membrane vesicles (OMV) (Chatterjee *et al.*, 2008; Ionescu *et al.*, 2014). OMVs release has been proposed as a strategy for *X. fastidiosa* to adjust attachment to surfaces facilitating its systemic spread within the plant host (Ionescu *et al.*, 2014). In this work, we have investigated if DSF could be found in OMVs produced by *X. fastidiosa* citrus (9a5c, Fb7) and grape (Temecula1) strains. Thus, OMVs were purified from bacterial culture supernatants and subjected to LC-MS analysis. For all three strains, we were able to detect XfDSF2 (2-cis-hexadecanoic acid). We also detected the presence of the saturated fatty acids C15:0 (CVC-DSF), C16:0 and C18:0 that may act as DSF antagonists (Ionescu *et al.*, 2016). These results indicate that OMVs might function as a DSF carrier. Moreover, we have also compared the protein composition of these OMVs. As expected, adhesion proteins and the lipase LesA, among other virulence proteins, were found in OMVs from all the three strains while CarB, a DSF degrading enzyme, was found only in Temecula1 OMVs. Funding for this research was provided by CAPES and CNPq.



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## Distribution and adaptation of *Xylella fastidiosa* in Californian grapevines

Vanhove M.\*, Sicard A., Almeida R.

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**Abstract:** *X. fastidiosa* has been endemic in California since the end of the nineteenth century, impacting the wine industry by causing Pierce's disease in infected grapevines (Stenger et al. 2010, Almeida and Nunney 2015). This study is sequencing 140 *Xylella* isolates from various localities in northern and southern California. Isolates have been collected from multiple hosts across a thirty years' period and will unveil the evolutionary history of the pathogen in the region. Results will yield insights on the selection forces acting within *Xylella* genome as well as the influence of climate in the distribution of *Xylella* subspecies within California. Adaptation of *Xylella* populations to these different Californian climates and ecological niches might be relevant in term of disease manifestation (Koide et al. 2004). The bacterium is well adapted to the Californian environment with the presence and the mixing of both *X. fastidiosa* subsp. *fastidiosa* and *multiplex* (Giampetruzzi et al. 2017). Inter-subspecific homologous recombination is common within this species (Giampetruzzi et al. 2017, Nunney et al. 2014) with the potential to lead to host plant shifts (Kandel et al. 2016). The study will investigate the impact of recombination (Coletta-Filho et al 2017) and other genetic elements such as plasmids and prophages on the evolution and adaptation of the different *Xylella* subpopulations present in California. An in-depth analysis of the pan-genome of the sequenced isolates will give insights on *X. fastidiosa* colonization, disease development and whether specific genes are associated with host-type specificity.

### Acknowledgment

This work has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N. 727987 "*Xylella fastidiosa* Active Containment Through a multidisciplinary-Oriented Research Strategy XF-ACTORS" and from the Marie Skłodowska-Curie program grant agreement N. 707013.

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Coletta-Filho HD, Francisco CS, Lopes JRS, Muller C & Almeida RPP. Homologous Recombination and *Xylella fastidiosa* Host-Pathogen Associations in South America. Phytopathology. 2017;107(3).

## Insights into the genome of the De Donno strain of *Xylella fastidiosa*

Giampetruzzi A.\*, Saponari M., Loconsole G., Zicca S., Essakhi S., Boscia D., Landa B.B., Chacon-Diaz C., Almeida R.P.P., Saldarelli P.

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**Abstract:** Genetic studies showed that the genotype of the olive-infecting strain of *X. fastidiosa* (Xf-De Donno) belongs to the sequence type ST53 within subspecies *pauca*, already reported to occur in Costa Rica. The analysis of single nucleotide polymorphisms (SNPs) and the study of the pan-genome of 27 available whole genomes were performed to determine the phylogenetic placement of Xf-De Donno. Maximum-parsimony and maximum likelihood trees constructed using the SNPs and the pan-genome data distinguished the subsp. *fastidiosa*, *multiplex*, *pauca*, *sandyi*, and *morus* and groups the Italian and three Costa Rican ST53 isolates in a compact clade that diverges from the South American *pauca* isolates. These findings were supported by the characterization of a conjugative plasmid shared by the four ST53 isolates and by the identification of a gene encoding a putative histidine kinase-like ATPase, which is not present in isolates from the subsp. *multiplex*, *sandyi* and *pauca*, but was detected in the four ST53 and ST21 isolates of the subspecies *fastidiosa* from Costa Rica. These data support the common and recent origin of the ST53 isolates. The complete and annotated genome sequence of the strain De Donno of *X. fastidiosa* was obtained by a combined Illumina and PacBio sequencing. This strain, recovered from an olive tree affected by Olive Quick Decline Syndrome (OQDS), when mechanically inoculated in different olive cultivars, caused symptoms identical to those observed in contaminated olive groves.

### Acknowledgment

This work has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N. 635646 "Pest Organisms Threatening Europe POnTE".

### Bibliography

Giampetruzzi A, Saponari M, Loconsole G, Boscia D, Savino VN, Almeida R, Zicca S, Landa B, Chacon Diaz C & Saldarelli P. (2017). Genome-wide analysis provides evidence on the genetic relatedness of the emergent.

Giampetruzzi A, Saponari M, Almeida RPP, Essakhi S, Boscia D, Loconsole G & Saldarelli P. 2017. Complete genome sequence of the olive-infecting strain *Xylella fastidiosa* subsp. *pauca* De Donno. Genome Announc <https://doi.org/10.1128/genomeA.00569-17>.

## Session 3 - *Xylella fastidiosa*: host plants and resistance

### Controlling Pierce's Disease with molecular and classical breeding

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**Abstract:** Breeding for Pierce's disease (PD) resistant winegrapes began many years ago but these efforts failed to combine high resistance with high quality. This lack of progress was a result of using *Vitis* species from the southeastern United States, which have a complex multigenic resistance. We discovered PD resistance that was inherited as a single dominant gene in *V. arizonica* and related species from the southwestern US and northern Mexico. We genetically and physically mapped the

resistance gene, *PdR1*, and used marker-assisted selection (MAS) to introgress *PdR1* into *V. vinifera* cultivars. Using aggressive training and MAS we were able to reduce the seed-to-seed cycle to 2 years and went through four modified backcross (BC) generations to obtain 97% *V. vinifera* hybrids. To avoid inbreeding depression, we used different *V. vinifera* cultivars for each BC generation. In the mBC4 generation we grew out large seedling populations. These were first selected for horticultural characters and high organoleptic quality, and then were repeatedly screened for the highest levels of *X. fastidiosa* resistance. The best were replicated and advanced to small-scale wine making (< 20L). To date, 5 advanced selections have been pre-released to grapevine nurseries for multiplication, and will be ready for commercial use in Summer 2020. Wines have been evaluated with professional tasting panels and judged to be very high quality. These vines are under testing across California in areas with extreme PD pressure, and several mBC3 and mBC2 selections are being tested in Florida, Alabama and Texas. Additional commercial scale plantings are planned for 2018. The next phase of the breeding program involves stacking additional resistance genes to produce PD resistant winegrapes with more broadly based resistance. We are also introgressing powdery mildew resistance from selections with a high percentage of *V. vinifera* and powdery mildew resistance from multiple sources.

#### Acknowledgment

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### Screening of olive germplasm for resistance to *Xylella fastidiosa* ST53: the state of the art

Boscia D.\*, Altamura G., Dongiovanni C., Giampetruzzi A., La Notte P., Loconsole G., Martelli G.P., Morelli M., Palmisano F., Potere O., Saldarelli P., Savino V., Susca L., Tavano D., Zicca S., Saponari M.

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**Abstract:** While different sources of natural resistance to *X. fastidiosa* have been described in grapevines and citrus, lack of solid information exists on possible sources of resistance/tolerance in the cultivars that characterize the wide olive germplasm. Preliminary field observations and laboratory analyses of a few cultivars, have shown that differential responses to *X. fastidiosa* infections exist. To confirm these preliminary findings, a large panel of olive cultivars is being specifically investigated. Currently, the screening procedure relies on field observations looking for symptomless subjects (trees of known cultivars/volunteer seedlings), mechanical inoculations, qualitative and quantitative diagnostic assays (ELISA & qPCR) and, in selected cases, comparative transcriptomic profiling. Field experiments include the planting of the target cultivars/selections in an infected area under high inoculum pressure. All the plots are located in the Apulia Region (Italy) in the demarcated infected area, surrounded by *X. fastidiosa* heavily affected olive groves. A first experimental plot was

established in April 2015 with 10 different cultivars, which was extended in 2016 to 49 cultivars, and will be further enlarged in 2017 with the addition of 40 new accessions. Other plots, comprising newly planted or grafted cultivars (over 260 cvs) have been also established, bringing to over 300 the total number of accessions under evaluation. Cvs Leccino and FS-17<sup>®</sup>, both expressing interesting traits of resistance, have already been identified.

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### ***Xylella fastidiosa* in olive trees in Brazil: survey, host susceptibility, and inoculation tests**

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**Abstract:** *X. fastidiosa* subsp. *pauca* – (ST16) was recently reported infecting olive trees - *Olea europaea*, in southeast Brazilian's states, Sao Paulo - SP and Minas Gerais - MG. Information like *X. fastidiosa* subsp. *pauca* spread through olive producing regions, symptoms associated with the *X. fastidiosa* subsp. *pauca* infection, potential infectivity of others sequence type (ST) of *X. fastidiosa* subsp. *pauca* in olive trees, and susceptibility of olive varieties to Xf infection are unknown. In this study, we are reporting preliminary results of research projects aiming better know the *X. fastidiosa* subsp. *pauca* – olive tree pathosystem. The rate of *X. fastidiosa* subsp. *pauca*-PCR positive olive samples (n = 89) from five different regions of SP state was 28%. Higher infection rate (71%) was observed for the samples (n=120) taken from 3 different geographic regions of MG state. Successful *X. fastidiosa* subsp. *pauca* isolation by using BCYE medium was obtained from most of PCR positive samples. Commercial olive varieties like Grappolo, Ascolana, Maria da Fé, Arbequina, Koroneiki, and 37 others varieties were found naturally infected by the bacteria. On the other hand, no natural infection was observed in Empeltre, Barnea, Zalmate, Negrao, Salome, and Gordal de Sevilla varieties, so far. The predominant leaves symptoms associated to *X. fastidiosa* infection start with scorching at the upper part of leaf followed by whole leaf scorching and general leaves scorching in the branch later on, which are similar to describe in southern Italy. Successful artificial infection was obtained by using the ST16 and ST66, off 6 others tested STs.

#### Acknowledgment

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## Study of plant growth conditions influencing *Xylella fastidiosa* disease in different plant species

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**Abstract:** We have studied the environmental conditions that have a role in regulating *X. fastidiosa*-induced diseases and host range. First, we report on a 5-year experiment in which four different plant species (*Quercus petraea*, *Prunus domestica*, *Vitis vinifera*, *Salix alba*) have been inoculated with a set of Xf strains, and are growing in insect-proof tunnels under Belgian climatic conditions. In *Vitis vinifera*, important spread of *X. fastidiosa* subsp. *fastidiosa* was already scored after 6 months, without expressing disease. All detection tests are repeated twice yearly. Preconditioning of *X. fastidiosa* cells for successful inoculation are also discussed. Second, we study plant growth conditions that change the chemistry of xylem sap, which is then extracted and used as the test medium for *X. fastidiosa* growth and expression of pathogenesis-related features. Two divergent conditions of temperature, water availability and fertilization, and their combinations, were installed in greenhouses to grow *Vitis vinifera*, *Nerium oleander* and *Salix alba*. After six months, xylem fluid was collected from all variables and this will be repeated for three years. These xylem sap samples are then inoculated with the relevant *X. fastidiosa* strains. We report on the *X. fastidiosa* behavior in these variable xylem conditions, such as growth forms (planktonic cells, aggregates, biofilms) and expression of cell wall-degrading activities. These data are then correlated with the xylem chemistry (pH, several organic and inorganic compounds) and the differential plant growth conditions

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## A global database on the host plants of *Xylella* spp.

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**Abstract:** *X. fastidiosa* was detected under field condition in Lecce province in Apulia (Italy) in October 2013. Since then it has been reported outdoors also in Corsica, South France and Balearic Islands. The European Food Safety Authority (EFSA) has produced urgent advices and risk assessment on *X. fastidiosa* to scientifically support the EU risk managers to prevent further introduction and spread of this pathogen. In particular, EFSA has developed and maintained a global database of the reported host plants of *Xylella* spp. A list of *Xylella* spp. host plant species was derived from an extensive literature search and included 359 plant species from 204 genera in 75 different botanical families (EFSA, 2016). Since then, EFSA has been tasked to periodically update the database in order to catch all new scientific developments published on the topic, including information on negative results of experimental infections and on plant varietal responses. Currently, a systematic approach is being applied for this update according to the EFSA guidance on systematic literature review (EFSA, 2010) in order to retrieve the studies, select the relevant ones, and collect the data. The new version of the *Xylella* spp. host plants global database will be completed by September 2017. The results obtained and the conclusions drawn will be presented.

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## Session 4 - *Xylella fastidiosa*: pathogen and disease control in the host plants

### Advances in controlling *Xylella fastidiosa* in the host plants

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**Abstract:** *X. fastidiosa* is responsible for diseases that affect different crops worldwide. One of them is CVC, the Citrus Variegated Chlorosis, which results from the xylem vessels occlusion by *X. fastidiosa* biofilm. CVC caused serious damage in Brazilian citrus production from 1998 until 2012 and after that its incidence started to decrease. The success in maintaining the citrus orchards with low level of CVC is due to the rigid control adopted by the farmers, which involves three practices: i. Production of seedlings in protected nurseries against insect vectors; ii. Pruning and eradication of severely diseased trees and iii. Chemical control of the insect vectors. However even though these measurements had maintained the citrus production in Brazil, the continuous period of drought and high temperatures in areas of citrus production represent a real risk for CVC increasing. In addition, the use of insecticides increased in the orchards because it is also used to control psyllids (vector of *Ca. Liberibacter*, causal agent of HLB) that also controls sharpshooters (*X. fastidiosa* insect vector), but the huge amount of insecticide has become economic and environmental unsuitable. For these reasons new and more sustainable practices are important to keep citrus as an important commodity in the Brazilian agribusiness. The team from Centro de Citricultura – IAC/Brazil has been working in three different approaches to control CVC: Breeding, transgenic plants and N-acetylcysteine (NAC) molecule. In relation to breeding a cross between *Citrus reticulata* (resistant) X *Citrus sinensis* (susceptible) was done where 260 hybrids were obtained and tested in different citrus producing regions. Among them eight hybrids were already selected as resistant to CVC and are now under evaluation for the agronomical traits. Another approach is the use of transgenic plants. For this, we are using genes from pathogen and from resistant plant (cisgenic method) to transform sweet orange plants. Transgenic plants carrying genes from *X. fastidiosa* were obtained in an approach named "pathogen confusion". The transgenic lines showing high level of CVC resistance were obtained and they have been propagated to be evaluated in field condition. Finally the third approach is the use of NAC, since previous results demonstrated that this molecule shows effectiveness as an antimicrobial compound and biofilm disrupting agent in *X. fastidiosa*. Here, we show advances in the study of the NAC effect in

plants. Two years of evaluation in field condition where NAC was applied in plants showing severe CVC symptoms, revealed that application of NAC to the roots was able to improve the production and fruit size in both diseased and healthy plants, indicating that this molecule has also a beneficial effect to the plant. Thus we investigated the antioxidative property of NAC and confirmed that treated plants showed a reduction of reactive oxygen species and induction of antioxidant enzymes. These results reinforce the potential of NAC to control *X. fastidiosa*. We believe that NAC application in healthy plants could help to avoid pathogen infection since plants with less oxidative stress are physiologically better and prompter to trigger defense responses against phytopathogens.

## Evaluation of field treatments to reduce the impact of *Xylella fastidiosa* infections in olive trees

Dongiovanni E.\*, Di Carolo M., Fumarola G., Ciniero A., Tauro D., Palmisano F., Silletti M.R., Pollastro P., Altamura G., Morelli M., Coletta-Filho H.D., de Souza A.A., Saldarelli P., Boscia D., Saponari M., Faretra F.

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**Abstract:** So far no effective field applicable therapeutic strategy exists to combat the severe diseases associated to *X. fastidiosa*. Therefore, various experimental applications to seek for a sustainable approach for the mitigation of the impact of the infections on olive trees were explored. In 2015-2016, four field trials were set in the infected area in southern Italy: (i) trial 1 consisted of an olive grove with medium to high incidence of disease symptoms; (ii) trials 2 and 3 consisted of olive groves with very low incidence of infected and symptomatic trees and (iii) trial 4 was a new plantation with 3-years old *Xylella*-free olive plants. Applications included (i) different elicitors of plant resistance (fosetyl aluminium, acibenzolar-S-methyl, COS-OGA,  $\sigma$ - $\beta$  Hairpin proteins, cerevisanae) and (ii) N-acetylcysteine (NAC), a mucolytic agent previously showed to have a beneficial impact in reducing symptoms associated to *X. fastidiosa* in citrus (1). Periodic surveys included (i) sampling for laboratory tests to determine the incidence of the infections; (ii) symptom scoring using 0-5 empirical scale of severity. The overall results showed that only the treatments with NAC, through endotherapy and/or complexed to organic substances added to the soil, determined a noticeable amelioration of the symptoms. None of the elicitors produced any measurable positive impact on the diseased trees. These preliminary encouraging results, prompted for further targeted experiments currently ongoing.

### Acknowledgment

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## The endophytic bacterial community of healthy and *Xylella*-infected olive sapwood

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**Abstract:** Endophytic bacteria are of biotechnological and agronomic interest as they promote plant healthiness by producing and secreting plant growth regulators, and antagonizing phytopathogens through the induction of resistance mechanisms, and the supply of nutritional elements. One of the factors that may influence the behavior of olive towards the 'quick decline syndrome' is the nature of the endophytic microbial community occurring in sapwood. Objectives of the research was to characterize the bacterial endophytic population occurring into the xylem of healthy and *Xylella*-infected olive trees by an isolation-dependent approach. Preliminary results indicate that under field conditions, the population level of cultivable endophytic bacteria is highly variable, being mainly affected by the host genotype, host age, and wilting severity. Among the different group are *Pseudomonas*, *Bacillus*, *Lysinibacillus*, *Pantoea*, *Microbacterium*, *Stenotrophomonas*, and *Methylobacterium* spp. Bacteria of the *Methylobacterium* genus occupy the same ecological niche of *X.*

*fastidiosa* subsp. *pauca*. It has been reported as potential biocontrol agent of the pathogen, being its population higher in citrus plant showing mild symptoms of variegated chlorosis. Further research is in progress to better characterize the different *Methylobacterium* strains, using both biochemical and molecular approaches, and to evaluate its activity in reducing the severity of olive quick decline syndrome.

#### Acknowledgment

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### Attempts to develop sustainable biocontrol strategies of *Xylella fastidiosa* infections in olive

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**Abstract:** *X. fastidiosa* is a plant pathogenic bacterium, which is causing the 'Olive Quick Decline Syndrome' (OQDS), on olive trees in the southern part of Apulia region. The knowledge of mechanisms regulating olive- *X. fastidiosa* interactions is fundamental to develop biocontrol strategies. In Pierce's Disease (PD) the pathogen virulence relies on a fine balance between motile cells, which move and proliferate in xylem vessels, and sticky cells forming a biofilm and responsible for vessels blockage and insect acquisition. This different behaviour is regulated by diffusible signalling factors (DSF), synthesised by a bacterial *rpfF*-gene, that regulate genes inducing biofilm formation. DSFs produced by the olive-infecting strain (CoDiRO) of *X. fastidiosa* were analysed by Gas Chromatography-Mass Spectrometry analysis. Preliminary results showed that a family of unsaturated fatty acids, with a chain length of 12-18 carbon atoms, is produced. They will be further characterised by nuclear magnetic resonance (NMR). These studies may be applied in a "pathogen confusion" strategy for mitigating *X. fastidiosa*-infections by altering DSFs level in planta. Pursuing this approach, a plant viral-based vector has been engineered to induce *rpfF* transient expression. This approach would make *X. fastidiosa* cells less motile and more sticky in xylem vessels, thus lowering their virulence. A model system is being evaluated to verify the DSF expression and accumulation directed by a viral-*rpfF* recombinant vector.

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## Antibacterial and plant defence elicitor peptides against *Xylella fastidiosa*

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**Abstract:** Synthetic antimicrobial peptides (sAMPs) offer great possibilities as novel compounds for the control of plant diseases. Libraries of AMPs have been prepared by solid phase chemistry and combinatorial approaches, by collaboration with the LIPPSO laboratory at the University of Girona. Leads from these libraries have minimal inhibitory concentrations in the range of common antibiotics, and some have additional activity as plant defence elicitors and cell-penetrating peptides. Several were effective in greenhouse experiments and field trials, against fireblight (*Erwinia amylovora*), bacterial blight of pear (*Pseudomonas syringae* pv. *syringae*), bacterial spot and canker of *Prunus* (*Xanthomonas arboricola* pv. *pruni*), and bacterial canker of kiwifruit (*P. syringae* pv. *actinidiae*), or against two diseases caused by phytoplasmas.

We are currently developing and evaluating antibacterial peptides against *X. fastidiosa* subspecies, in the XF-ACTORS EU project (727987), in collaboration with the IAS-CSIC. The sAMPs BP100, BP15, BP178 (linear peptides) and BPC194 (cyclic decapeptide) have been synthesized and are being tested *in vitro* for their bactericidal activity against *X. fastidiosa* subspecies. A selection of sAMPs will be evaluated on olive plants for phytotoxicity. Finally, evaluation of lead sAMPs will be performed for their ability to suppress *X. fastidiosa* infections and symptoms by foliar application and trunk injection to young olive seedlings.

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## Session 5 - Vectors and epidemiology

### Diversity of xylem-feeders and their role in epidemiology of diseases caused by *Xylella fastidiosa*

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**Abstract:** An increasing number of diseases of fruit crops and ornamental plants have been associated with the xylem-limited bacterium *X. fastidiosa*, which can be transmitted by several species of sharpshooters and spittlebugs. A large diversity of sharpshooters and spittlebugs has been found in association with affected crops, but for most of them the role as vectors of *X. fastidiosa* is unknown. Vector relevance depends not only on ability to transmit the pathogen, but also on ecological and behavioral characteristics in consonance with other epidemiological components of the disease, such as source plants, pathogen dynamics in these hosts, and favorable environmental conditions. For citrus variegated chlorosis, infected citrus trees represent the most important (if not the only) source of inoculum, and four sharpshooter species that commonly feed and reproduce on citrus trees are considered key vectors. For less studied *X. fastidiosa* pathosystems, e.g. plum leaf scald and the newly discovered olive leaf scorch in Brazil and Argentina, there is limited information on vectors and inoculum sources. Weeds, shrubs and trees in the spontaneous vegetation should be investigated as hosts of *X. fastidiosa*. Sharpshooters and spittlebugs inhabiting these plants should be tested as natural carriers of *X. fastidiosa* and for their competence for transmission. Determining what vector species and inoculum sources are relevant for pathogen spread is basic to establish disease management strategies.

### Phenology population dynamics and host plants of *Philaenus spumarius* in Italian olive groves

Bodino N.\*, Plazio E., Picciau L., Cavalieri V., Dongiovanni E., Di Carolo M., Tauro D., Volani S., Salerno M., Russo V., Porcelli F., Gilioli G., Bosco D.

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**Abstract:** *Philaenus spumarius* is the vector of the CoDiRO strain (subsp. *pauca*) of *X. fastidiosa* in the Apulian olive orchards. Detailed knowledge on ecology and phenology of *P. spumarius* is needed to design control measures aiming at slowing disease progression and assessing the risk in non-infected areas. Open field surveys are conducted in olive orchards in Liguria and Apulia Regions of Italy. Structured population dynamics, host-plant association, seasonal movement between crop and wild species, were investigated by field surveys from March 2016 and are scheduled until the end of 2017. Nymph population in the grass cover was estimated by direct counting in thirty random sampling units (SU) in each olive orchard. Life stage of the insect, host-plant and its phenological stage were recorded. Adults were collected with sweeping net on three different vegetational components: grass cover (30 SU), olive canopy (20 SU), and alternative woody hosts (10 SU). All samplings were conservative to avoid disturbance to spittlebug population. Nymph population peaked in the second half of April in Liguria and at the end of March in Apulia. Early instars were frequently found on plants with basal rosettes, mainly Asteraceae, while later instars moved to upper part of the plant, exploiting many more plant species. Adults show host-shifting during the season as they move from herbaceous hosts to woody plants in the summer and come back to grass cover at the end of the summer.

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## Seasonal pattern hosts and abundance of the potential vectors of *Xylella fastidiosa* in Mallorca

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**Abstract:** The plant pathogen *X. fastidiosa* (Proteobacteria: Xanthomonadaceae) was detected in the Balearic Islands (Mallorca, Menorca and Ibiza) in October 2016. Since then, a surveillance of the potential vector species that are included in the suborder Cicadomorpha has been conducted. The sampling was carried out in different crops from March 2017, including olive, almond, citrus and vineyards of Mallorca, as well as on the herbaceous vegetation of each sample site. Immatures and adults were sampled biweekly in each plot by using general techniques, such as direct observation, sweeping net and vacuum. Collected adults were identified to species level and mouth parts and cibarium were preserved for molecular detection of *X. fastidiosa* by PCR (analysis still ongoing). Preliminary results showed that several species of the family Aphrophoridae were the most frequent and abundant in the selected orchards, particularly *Neophilaenus campestris* and *Philaenus spumarius*. Nymphs of these species were found from early to late April only and showed preference for particular herbaceous plant species. Adults were also collected from early April and the sampling is still ongoing. We present data on the presence of potential adults vectors in herbaceous plants and crop trees for each month from March to October 2016 and discuss about the impact on *X. fastidiosa* transmission in the Balearic islands.

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## Significance of xylem feeding Auchenorrhyncha in orchards and vineyards in Germany

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**Abstract:** In the frame of the European XFactors-project, a study is carried out on the presence, host affiliation and life history of xylem feeding Auchenorrhyncha in Germany. The objective of this work is to acquire data on the potential risk posed by those species for the establishment and spread of *X. fastidiosa* under central European conditions. While the most likely pathway of the pathogen into this region are infected ornamental plants it has to switch to woody plants to survive cold winter conditions and get established in permanent reservoir plants. Special emphasis is therefore placed on polyphagous xylem-feeding species common in relevant cultures like orchards and vineyards, their host affiliation throughout the vegetation period and their feeding behavior on both herbaceous and woody hosts. The results of this study will improve the knowledge on potential vectors of *X. fastidiosa* in Central Europe and support rational risk analyses for the establishment of the pathogen in this environment. Results of the first season of the study will be presented.

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## The distribution and phenology of five potential vectors of *Xylella fastidiosa* in Belgium

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**Abstract:** The distribution and phenology of five potential vectors of *X. fastidiosa* in Belgium (*Cercopis vulnerata*; *Cicadella viridis*; *Philaenus spumarius*; *Aphrophora alni*; *Aphrophora salicina*) have been analysed by field surveys, a systematic search in the collections of the Royal Institute of Natural Sciences and a review of a Belgian online faunistic database, Observations.be. The five species are present and often abundant in all the sites visited. The adults of *C. vulnerata* are only briefly present in May, whilst the adults of the other species have a more protracted presence. All species except *C. viridis* are univoltine. All species except *C. vulnerata* overwinter as eggs. The significance of these results regarding the epidemiology of *X. fastidiosa*, should it enter Belgium, is discussed.

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## Potential insect vectors of *Xylella fastidiosa* in the United Kingdom

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**Abstract:** The bacterium *X. fastidiosa* resides in plant xylem tissues and Auchenorrhyncha bugs (Hemiptera) that feed on the xylem are potential vectors. Knowledge of these potential vectors is essential for developing risk assessments, contingency plans and management options. A review of potential vectors in the UK identified 18 species in four families (Aphrophoridae 9 spp.; Cercopididae 1 sp.; Cicadellidae 7 spp.; and Cicadidae 1 sp.). However the situation is more complicated as phloem-feeders, such as species of the subfamily Deltocephalinae, can occasionally consume from, or at least be in contact with, xylem tissues and take up the bacterium, although none have been shown to be vectors (Elbeaino et al., 2014; Pompon et al., 2011). In Europe only *Philaenus spumarius* (meadow

spittle bug) is confirmed to be a vector (EFSA, 2015). It is very common and widespread in the UK and broadly polyphagous (100+ mainly herbaceous hosts). It occurs in a wide range of habitats and is occasionally recorded feeding on trees, especially in late summer in dry years. The majority of the other potential vectors in the UK are most commonly recorded in species-rich grasslands and marshes and are therefore less likely to be found at or near commercial plant nurseries. In order to determine the frequency of potential vectors in the UK a citizen science survey will be conducted during 2017, and the results presented.

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### **Preliminary evaluation of different insecticides against *Philaenus spumarius***

Dongiovanni E.\*, Di Carolo M., Fumarola G., Tauro D., Cavalieri V., Altamura G., Saponari M., Porcelli F.

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**Abstract:** The meadow spittlebug *Philaenus spumarius* is the first ascertained vector of *X. fastidiosa* in Europe, being responsible for the current Xylella-epidemics in olive orchards in Apulia (southern Italy). Preliminary studies revealed that adults acquire and transmit the bacterium after their migration from the ground vegetation to the olive canopies during the dry season (late spring-summer). An effective suppression of the vector populations requires an integrated pest management strategy against nymph and adult stages. Lack of registered and authorized formulations for the control of this insect on olives, prompted for setting up field trials on comparative efficacy of different formulations. We tested the possibility of applying different insecticides, natural or inert substances (extract of citrus oil, kaolin, zeolite) and synthetic products (deltamethrin, buprofenzin, imidacloprid) to suppress the juveniles. Whereas, a total of twelve different formulations based on active compounds belonging to different chemical/ organic families and with different mechanisms of action and translocation were tested for the control of the adults. Neonicotinoids and pyrethroids showed the highest efficacy both for the control of the juvenile stages and the adult populations. The results obtained in our work provide first evidences on the efficacy of different formulations for the control of *P. spumarius* toward the implementation of containment strategies for *X. fastidiosa* in olive groves

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### ***Zelus renardii* an assassin bug candidate for *Philaenus spumarius* biocontrol.**

Salerno M., Russo V., Sefa V., Lamaj F., Basher N., Verrastro V., Porcelli F.\*

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**Abstract:** Spittlebugs (Hemiptera; Aphrophoridae) natural enemies appear to be a not so effective guild. Olive orchard now invaded by *X. fastidiosa pauca* OQDS strain or threatened of, do not constitute the exception. *Zelus renardii* (Zr) (Hemiptera; Reduviidae) originate in Nearctic but spontaneously acclimated in Europe. We attempted the assassin bug breeding from 2015 starting with *M. gladiata* adults and nymphs, and have had the opportunity to offer many preys belonging to several insect orders to the assassin bug. Adult *Z. renardii* demonstrated to successfully attack and feed on almost all the offered insects, including large Coccinellidae as *Harmonia axyridis* (Pallas, 1773) (the Asian ladybeetle) and comparatively enormous *Gromphadorhina portentosa* (Schaum, 1853) (Blattodea Blaberidae; the Madagascar hissing cockroach) juveniles. Recently we offered to Zr for food adult, and juveniles of several phytophagous Hemiptera Taxa, including *Philaenus spumarius*, all of those have successfully preyed. Evidence suggest that Zr may serve as a biological control agent in olive orchards, in an inoculative or inundative program if bred in sufficient number. To have the opportunity we systematically tried several different dietary regimes all functional to a Zr mass-breeding program: from living insect preys to a purposely formulated meridic artificial diet. Revealing our about a one-year long experience, we discuss predator bionomics about a full artificial breeding possibility.

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## Session 6 – Detection and identification

### From transnational research collaboration to regional Standards: the EPPO diagnostic protocol for *Xylella fastidiosa*

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**Abstract:** In 2013 the EPPO Secretariat received the official report of *X. fastidiosa* by the National Plant Protection Organisation of Italy. A regional Diagnostic Protocol on the bacterium had been published in 2004 but it focussed mainly on *Citrus* spp. and *Vitis*. Because of the significant changes in the bacterium's geographical distribution and its list of plant host species, the revision of the EPPO Diagnostic Protocol was considered a priority; an expert working group was established and the EPPO Standards on *X. fastidiosa* was published in September 2016.

As the scientific knowledge on the bacterium, its vectors and host species has advanced and diagnostic tests have been and are being developed and validated in the framework of national and transnational collaborative research projects, work has started on a further revision of the protocol. The presentation will focus on a comparison of the different versions of the EPPO Diagnostic Protocol on *X. fastidiosa* and will show how research evidences support diagnostic activities and more broadly, regional policy.

### First international proficiency testing for laboratory performance on *Xylella fastidiosa* detection

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**Abstract:** A proficiency test (PT) to evaluate the performance of laboratories involved in molecular and serological detection of *X. fastidiosa* was carried out in early 2017; 35 laboratories from EU/non-EU Countries tested 4 different methods to purify DNA, conventional and qPCR assays, and 2 ELISA tests. The number of resultant positive agreement/negative agreement/positive deviation/negative deviation was used to determine the laboratory performance (i.e. accuracy 100%). The overall results showed that all laboratories were able to correctly diagnose *X. fastidiosa* in the blind samples



containing the highest *X. fastidiosa* concentrations, whereas the performance of several laboratories was negatively affected by the lack of detection in the samples with the lowest concentrations, both through molecular and serological tests. Accuracy level of 100% (laboratory conformed to the PT) was successfully recovered in the majority of the laboratories performing qPCR and PCR assays on DNA purified using at least 2 of the 4 tested protocols. The use of automated platform ensured higher laboratory performance. As expected, results of the ELISA tests generated lower performance values in the majority of the laboratories, due to the lack of detection of positive samples containing the lowest the bacterial concentration. This study provides a good overview on the laboratory performance for the diagnostics currently used in the EPPO countries and indicate useful improvements that laboratories can adopt to achieve a better performance.

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### Towards an efficient sampling procedure for early detection of *Xylella fastidiosa* in asymptomatic plants

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**Abstract:** In the past, *X. fastidiosa* was restricted to a number of countries through the America's (Almeida and Nunney 2015). This situation has been dramatically altered in Europe as *X. fastidiosa* has been found now in various host plants in several European countries. Movement of plants for planting is considered to be the most important pathway for entry of the pathogen into Europe, especially when present without symptoms (EFSA PLH Panel 2015). The significance of this pathway is enhanced by the enormous host range of *X. fastidiosa* and the high volumes of plants for planting used to be imported from countries where *X. fastidiosa* was known to occur. Currently there is an urgency for guidelines for sampling, specifically when asymptomatic *X. fastidiosa* infections can easily escape laboratory testing, due to its heterogeneous distribution in plant, that may lead to false-negative results. Experiments have been initiated towards an efficient sampling for early detection of *X. fastidiosa* in asymptomatic plants under confined conditions, by simulating different levels of infection in plants and sampling at different % and different sample size. Strains PD 7202 (ST 53) and PD 7211 (ST 73) belonging to *X. fastidiosa* subsp. *pauca* and originating from *C. arabica* are used (Bergsma-Vlami et al 2017). Particular attention is given to *P. myrtifolia*, *P. avium*, *P. domestica*, *N. oleander* and *C. arabica*. In our system, pre-defined *X. fastidiosa* inoculum per unit (f.w of petioles/midribs in g) is being mixed with healthy units at different infection levels and analysed based to EPPO diagnostic standard (EPPO 2016). Preliminary results will be presented.

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## Improving the typing of *Xylella fastidiosa* directly from plant material

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**Abstract:** Detection of *X. fastidiosa* directly from plant material is a common practice as a screening test for epidemiosurveillance purposes. MultiLocus Sequence Analysis and Typing (MLSA/MLST) methods are amongst the most widely used genotyping methods for assessing the global epidemiology of various plant pathogenic bacteria including *X. fastidiosa*. MLSA is used to assign strains to one of the known *X. fastidiosa* subspecies and MLST for typing strains and make inference of their origin. Both methods are traditionally used on isolated strains. Nonetheless, because it is time-consuming and sometimes difficult to obtain isolates of *X. fastidiosa* from plant samples as a consequence of an intrinsically poor isolation rate and the fastidious nature of the pathogen, being able to type *X. fastidiosa* in plant samples is a priority for epidemiological purposes. Application of the initial protocol on DNA extracted from plant material proved limited efficiency. The aim of our study was to test the impact of various modifications of the protocol in order to improve the efficiencies of DNA extraction and target amplification rate. We also tested the interest of reduced typing schemes. The direct identification of *X. fastidiosa* infecting plant material face however some limitations that will be discussed.

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## Real-time LAMP rapid diagnostic method for *X. fastidiosa* in plant material and insect vectors

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**Abstract:** *X. fastidiosa* is a gram-negative insect-vectored bacterium has been recently detected in Italian olive trees severely affected by Olive Quick Decline Syndrome (OQDS). ELISA tests and Polymerase Chain Reaction (PCR) assays were largely used in the monitoring campaign of this pathogen. The aim of this study is to evaluate the new real time Loop-mediated isothermal amplification (LAMP) system for the detection of *X. fastidiosa* in host plants and insect vectors. The new detection system is composed of a portable instrument (icgene mini) and a ready to use diagnostic kit denominated "Xylella Screen Glow" (Enbiotech s.r.l.- Italy). Specificity and sensitivity of Xylella Screen Glow kit was compared with PCR and real-time quantitative PCR assays. Three different DNA extraction protocols and typologies of infected materials were also tested. The real-time LAMP system showed high specificity and a sensitivity as compared to the real-time qPCR and PCR assays. These results showed that "Xylella Screen Glow" kit with icgene portable LAMP instrument is highly



sensitive and suitable for *X. fastidiosa* detection directly in the field. Therefore, this system could be applied on site, with high sensitivity and readability to prevent the movement of infected materials from *X. fastidiosa* contaminated area to the laboratories located in free areas; other possible application could be in quarantine station to intercept any infected material before crossing border and protect from a country from such dangerous quarantine pathogen.

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#### Bibliography

[YASEEN, Thaeer et al. On-site detection of \*Xylella fastidiosa\* in host plants and in "spy insects" using the real-time loop-mediated isothermal amplification method. \*Phytopathologia Mediterranea\*, \[S.l.\], v. 54, n. 3, p. 488-496, sep. 2015. ISSN 1593-2095. Date accessed: 20 May. 2017. doi:10.14601/Phytopathol\\_Mediterr-15250.](#)

## Session 7 – From field detection to disease dynamics

### Detecting *X. fastidiosa* with hyperspectral remote sensing: findings from two years of airborne campaigns in Puglia

Zarco-Tejada P.J.\*, Calderon R., Beck P.S.A., González-Dugo V., Montes-Borrego M., Hornero A., Landa B.B., Boscia D., Susca L., Navas-Cortes J.A.

*\*Joint Research Centre, European Commission*

**Abstract:** A remote sensing campaign carried out in Puglia in summer 2016 collected hyperspectral and thermal images of ca 200,000 olive trees at sub-meter resolution. In the 1200 ha study area within the *X. fastidiosa* infected zone, 3500 trees were simultaneously evaluated in the field for severity of *X. fastidiosa* symptoms. The hyperspectral sensor used in this experiment acquired data in the visible and near-infrared spectral region (400-885 nm) with 260 bands of 6.5 nm FWHM at 1.85 nm/pixel and 12-bit radiometric resolution. The sensor was radiometrically calibrated in the laboratory, and images atmospherically corrected to obtain surface reflectance using total incoming irradiance measured in the field. The high resolution hyperspectral and thermal imagery acquired over the orchards allowed the delineation of individual trees using object-based crown detection algorithms. Crown temperature and hyperspectral indices were calculated for each tree to classify disease severity levels using different machine learning algorithms, including linear discriminant analysis, support vector machines and neural networks. The success and applicability of these early detection methods to other areas will be discussed in the context of a new airborne campaign planned in July 2017 over *X. fastidiosa* infected zones in Mallorca. In this new airborne campaign the impact of *X. fastidiosa* symptoms on crown reflectance will also be evaluated in the 800-1700 nm spectral region.

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### Preliminary results on the canopy distribution of *Xylella fastidiosa* in the Apulian olive cultivars

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**Abstract:** Pathogen detection in the Italian outbreak area of *X. fastidiosa* is carried out using samples from symptomatic olive twigs and leaves. Conversely detection from asymptomatic trees is difficult due to the uneven pathogen distribution in olive cultivars. In this study the incidence and canopy

distribution of the infection is under assessment in 4 olive cultivars (EU H2020 project 'Xf-Actors') grown in a field (about 1.5 Ha) in the infected area of Apulia. All trees were tested in March for the presence of *X. fastidiosa* using real time LAMP (Loop Mediated Isothermal Amplification). The highest infection rate was detected in 'Cellina di Nardò' (74%) and 'Cima di Melfi' (76%), whereas the lowest was recorded in 'Leccino' (61%) and 'Frantoio' (63%). Based on these results, 5 trees were selected from 'Cellina di Nardò' (highly susceptible) and 'Leccino' (less susceptible). About 4 twigs were randomly collected from 3 levels of the asymptomatic sector of the canopy and each twig was divided in 3 portions. About 180 samples/cultivar are collected 3 times from March to July and tested by DTBIA and real time LAMP. After the first 2 samplings a preliminary difference was noticed on the infection distribution: the whole canopy in the case of 'Cellina di Nardò' and the lower part of the canopy in the case of 'Leccino'. However, final results will be achieved after the 3rd sampling in July and data analysis by Univariate and Multivariate ANOVA test.

#### Acknowledgment

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## Spatial and temporal dynamics of Olive Quick Decline Syndrome in orchards in Puglia, southern Italy

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**Abstract:** The spatial dynamics of Olive Quick Decline Syndrome (OQDS) caused by *X. fastidiosa* subsp. *pauca* were determined in 20 olive plots in a selected olive growing area within the infected zone of the Lecce province, Puglia by assessing disease incidence (DI) and severity (DS) (0-5 rating scale) in June, 2016. Eight of the 20 plots with a wide range of initial DI and DS values were selected to assess the spatial and temporal dynamics of the OQDS and were evaluated in October 2016 and February 2017. Analyses are in progress and include the use of the Spatial Analysis by Distance Indices (SADIE) (Perry et al., 1999) to quantify the spatial pattern of OQDS. Spatial association between time periods was determined by the SADIE association index (Winder et al., 2001). Spatial pattern of symptomatic trees was estimated as regular (22.2% of plots), non-aggregated (33.3% of plots) or aggregated (44.4% of plots). Overall, both, DI and DS increased in the second and third assessments as compared to the initial score in June 2016 by  $28.7 \pm 4.2\%$  and  $2.02 \pm 5.1\%$  in DI, respectively; and by  $0.53 \pm 0.27$  and  $0.04 \pm 0.06$  in DS, respectively. Spatial pattern was characterized by the occurrence of several clusters of diseased trees. Increasing clustering over time was indicated by stronger values of the clustering index and the increase in patch cluster size. Spatial association was also found in the clustering of diseased trees between evaluation periods.

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## Insights into the spatio-temporal spread of *Xylella fastidiosa* in south-eastern Italy

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**Abstract:** *X. fastidiosa* causes serious diseases in a broad range of woody plants (EFSA 2015). The pathogen is xylem-limited and vectored by insects. Several subspecies have been described, differing in their host range, aggressiveness, and vectors. The movement of infected plants and vectors are considered the main pathways of introduction. In 2013, olive quick decline, caused by *X. fastidiosa* subsp. *pauca*, was first reported in Apulia in south-eastern Italy (Martelli *et al.* 2016) and *Philaenus spumarius* was confirmed as the main vector. Spatio-temporal analysis can inform about the epidemiological factors driving disease distribution and may assist to limit further spread. A mechanistic model was recently developed for the early stages of *X. fastidiosa* invasion in Apulia (White *et al.* 2017). In the present study, spatio-temporal models were fitted to the distribution of *X. fastidiosa* in Apulia from 2013 to 2016. Different sampling strategies were followed each year, so the analysis was focused only in the Salento peninsula where a more regular sampling was observed. Bayesian inference by INLA (Rue *et al.* 2009) was used and the geostatistical effect was implemented by the SPDE approach (Lindgren *et al.* 2011). Results confirmed the high rate of disease spread under favorable environmental conditions, absence of effective vector control, and presence of highly susceptible hosts.

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## Modeling and estimating the dynamics of *Xylella fastidiosa* based on French surveillance data

Soubeyrand S.\*, Martin O., Fernandez-Diclo Y., Coville J., Bonnefon O., Abboud C., De Jerphanion P., Saussac M., Hendrikx P., Lannou C.

*\*INRA, Institut National de la Recherche Agronomique, Avignon (FR)*

**Abstract:** Since the detection of *X. fastidiosa* in 2015 in Corsica, France, a rather intense surveillance of infections has been carried out and has generated a total of nearly 18000 samples, 5% of which

have been analyzed as positive. Using these data and statistical inference techniques, we have developed different models of the temporal and spatio-temporal dynamics of *X. fastidiosa* in southeastern France. In this talk, we will present two approaches: the first one is based on a stochastic temporal model allowing us to provide estimates of the introduction date in Corsica and the hidden component of the epidemic; the second one is based on a multi-component system of differential equations allowing us to reconstruct the regional spatio-temporal dynamics of the pathogen and the vectors, and to estimate epidemiological parameters. Both approaches can be used to draw forward projections of the epidemic under various control strategies and, therefore, could be used in the future for decision making.

#### *Aknowledgment*

This work has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N. 727987 "*Xylella fastidiosa* Active Containment Through a multidisciplinary-Oriented Research Strategy XF-ACTORS" and from INRA-DGAL project 2100067.

## Session 8 – Surveillance and control: from modelling to stakeholders analyses

### Developing surveillance strategies for *Xylella fastidiosa* in the Mediterranean region

Mastin A.\* and Parnell S.

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**Abstract:** Surveillance occupies a central position in disease management, being integral to all stages of pathogen mitigation: initial detection; quantification of pathogen extent in the population or region; monitoring of control schemes; and declaration of pathogen freedom. We have previously shown that surveillance and control strategies for many (if not all) of these pathogen scenarios can be improved by accounting for the biology of the pathogen; the environment (such as the distribution of host species); the diagnostic methods used for detection; and logistical issues such as cost and accessibility (Parnell *et al.* 2009, 2010, 2012, 2014, 2015; Alonso Chavez *et al.* 2016). However, surveillance schemes are often implemented with minimal consideration of these issues, resulting in suboptimal performance. Here, we show how to develop effective surveillance strategies for *X. fastidiosa* in the Mediterranean region - focussing on how simple biologically-informed "rules of thumb" can be used to target early detection surveillance activities in order to exploit heterogeneities in pathogen spread, and how computational methods can be used to optimise case finding during the early stages of an epidemic. Further work will investigate the effect of linking surveillance strategies more explicitly with control strategies, in order to ensure that an integrated approach towards pathogen mitigation is achieved.

#### *Aknowledgment*

This work has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N. 727987 "*Xylella fastidiosa* Active Containment Through a multidisciplinary-Oriented Research Strategy XF-ACTORS".

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## Modelling the spread and control of *Xylella fastidiosa* in the early stages of invasion in Apulia

White S.\*, Bullock J. and Chapman D.

\*NERC Centre for Ecology & Hydrology, Wallingford (UK)

**Abstract:** Since the initial outbreak, *X. fastidiosa* has invaded vast swathes of olives in the Apulian Region, southern Italy, and is of great concern throughout Mediterranean basin. Therefore, predicting its spread and estimating the efficacy of control are of utmost importance. As data on this invasive infectious disease are emerging, we have developed a spatially-explicit simulation model for *X. fastidiosa* to provide guidance for predicting spread in the early stages of invasion and inform management strategies. The model qualitatively and quantitatively predicts the patterns of spread. We model control zones currently employed in Apulia, showing that increasing buffer widths decrease infection risk beyond the control zone, but this may not halt the spread completely due to stochastic long-distance jumps caused by vector dispersal. Therefore, management practices should aim to reduce vector long-distance dispersal. We find optimal control scenarios that minimise control effort while reducing *X. fastidiosa* spread maximally—suggesting that increasing buffer zone widths should be favoured over surveillance efforts as control budgets increase. Our model highlights the importance of non-olive hosts which increase the spread rate of the disease and may lead to an order of magnitude increase in risk. In this presentation we will also highlight future model developments in the XF-ACTORS project (727987).

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White, Steven M., James M. Bullock, Danny AP Hooftman & Daniel S. Chapman. "Modelling the spread and control of *Xylella fastidiosa* in the early stages of invasion in Apulia, Italy." *Biological Invasions* (2017): 1-13. <http://rdcu.be/ryLf>

## Evaluating containment options for a *Xylella* outbreak in Andalusia through network analysis

Strona G., Castellano C., Fattorini S., Beck P. S. A\*

\*Joint Research Centre, European Commission

**Abstract:** The lack of data on pathogen spread complicates the fight against *X. fastidiosa* in Europe. Our previous work showed how the spatial arrangement of host plants can be used to elucidate management options in spite of such knowledge gaps; we generated networks of potential infection pathways from assumed vector dispersal ability and a map of olive orchards to assess the magnitude of the *Xylella* outbreak in Puglia. Here, we build on this technique to investigate scenarios of *Xylella* reaching Andalusia and causing OQDS. We compared the relative performance of two contrasting responses: 1) a preventive immunization strategy which encompasses different potential a priori measures to keep orchards uninfected, and 2) a quarantine strategy enacted at different stages of a hypothetical outbreak (a posteriori). Our results indicate that, from an epidemic perspective, preventive immunization is the best option to protect Andalusia from an OQDS epidemic. A posteriori quarantine action can only be successful if it is rigorously implemented at the first infection event; it should be applied to all hosts within the vector's maximum flight distance and before any quarantined



node has become infectious, thus requiring a reliable early detection program. We evaluate how the efficacy of quarantine changes as the epidemic progresses, and how it compares to preventive immunization as well as to non-intervention. We discuss the relevance of our findings for OQDS prevention on mainland Spain.

#### Bibliography

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### Understanding olive growers' intention to participate in *Xylella fastidiosa* control in Apulia, Italy

Ferilli F., Rickebusch S., Mourits M.\*

*\*Wageningen University, Wageningen (NL)*

**Abstract:** In this study the Theory of Planned Behaviour was used to study psychological and sociodemographic factors associated with olive growers' intentions to participate in the control of *X. fastidiosa* in southern Italy. Questionnaires were distributed to 96 growers in the Lecce province, which was considered to be completely infected by *X. fastidiosa*. Results indicated that 97% of the olive growers intended to report a suspected infection to authorities, while 84% showed a high intention to prune healthy olive trees to slow down the disease spread. Only 26% indicated to take part in phytosanitary treatments against the disease's vectors, although 82% agreed with applying soil tillage and weeding to reduce the vector population. The intention among growers to participate in the uprooting of infected trees at a compensation amount equal to the olive tree market price was limited to 38%. In case of no compensation, only 30% agreed with the measure of uprooting. Olive growers' intention was influenced mainly by their attitude and perceived behavioural control towards the application of the evaluated control measure. Education and income resulted among the most influencing sociodemographic variables, with high level of income (> 25,000 Euro a year) and high level of education associated with higher odds of participating in the control measures. Targeted information campaigning and subsidizing low incomes could therefore foster the rate of application of control measures.

#### Acknowledgment

This research has been performed by Franco Ferilli as part of his MSc programme Environmental Sciences at the Wageningen University, under the supervision of dr Sophie Rickebusch and dr, Monique Mourits.

Dr Sophie Rickebusch is a member of the Environmental System analysis group of the Wageningen University and an expert in ecosystem modelling. Dr. Monique Mourits works at the Business Economics group of the same university and is experienced in socio-economic impact analyses of regulated plant and animal diseases. She is currently involved in the Horizon 2020 project PONTE, focussing among others on the risk assessment of *Xylella fastidiosa*.

### Results from the automated media monitoring on *Xylella fastidiosa* in the Medisys platform

Linge J.\*, Mannino M. R.\*\*, Mayr M., Stancanelli G.

*\*Joint Research Centre, European Commission, \*\*Animal and Plant Health Unit, European Food Safety Authority, (IT)*

**Abstract:** Following a request from the European Commission (EC), EFSA provides monthly newsletters summarising media and literature monitoring. The aim is to identify relevant information on pests that might be of concern to the EU. Currently 234 pests are monitored from the EU and EPPO quarantine and alert lists. The media are screened using the MEDISYS (Medical Information System) platform. MEDISYS was extended from monitoring of human health to plant health threats in an EFSA-funded project in collaboration with the Joint Research Centre of the EC, the University of Lleida and the Institute for Food and Agricultural Research and Technology (Spain). The items coming from the sources are filtered using categories with given keywords to identify their plant health

relevance. In total, MEDISYS retrieves and analyses more than 200,000 items per day. Each monthly newsletter contains: a summary of the main issues, the links to the articles, the publication date, the first lines of the original articles, the automatic English translation of the title and of the first lines. Links to machine translations into English, French and German are available. The media and literature monitoring shows that *X. fastidiosa* is a highly discussed topic. Many items refer to detection, prevention and control. The difficulty of implementation of measures in the EU demarcated areas and the use of resistant or tolerant olive tree varieties are other highly discussed topics.

## Stakeholders' perception of *Xylella fastidiosa* disease: the case of Apulia region in southern Italy

Bogliotti C.\*, Ladisa G., Calabrese J., Cataldi G., Zdruli P.

*\*CIHEAM-IAM Mediterranean Agronomic Institute of Bari, Bari, (IT)*

**Abstract:** *X. fastidiosa* was discovered first near Lecce, Italy in 2013. Since this initial outbreak, the disease has expanded in more than 23,000 ha of olives in Apulia associated with significant socio-economic and environmental damages. At regional level, four zones identified as i) infected, ii) buffer, iii) containment and iv) safe/clean are confined but mitigation actions are on-going only in the first two zones. They include eradication of infected trees and the creation of a buffer zone to contain further outbreaks. Local stakeholder reaction to these actions have been mixed and largely contested by farmers, strongly opposing tree cutting. However, a systematic approach for risk assessment, risk management and prevention requires the involvement of all stakeholders. To reach this goal, a comprehensive stakeholder analyses was conducted in all four zones by completing a detailed questionnaire through 120 interviews with diverse groups of stakeholders representing regional and local decision makers, farmers, agronomists, and extension service agents. Data collection and elaboration is still on-going but preliminary results show that farmers in the infected area are very much concerned about the economic losses caused by the disease and no quick solutions to the problem while those in the other three zones are not fully aware of the potential risks. Nevertheless, all emphasise the need for technical assistance and post-emergency actions that include land reclamation and change.

### Acknowledgment

This work has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N. 727987 "Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy XF-ACTORS".

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## Final Session

### Agricultural research and innovation in Horizon 2020: Plant health in the SC2 Work Programme 2018 – 2020

Ganci P. E.\*

*\*European Commission, DG AGRI*

**Abstract:** Agricultural research and innovation have a major contribution to make in ensuring food security in the long term, addressing environmental sustainability and resilience and boosting the sustainable growth of rural territories. However agriculture is facing mounting pressure due to the increasing number and frequency of new and re-emerging pests and pathogens facilitated by intensification, globalisation, trade and climate change. European agriculture needs to cope with these threats to avoid trade disruptions and ensure consumer confidence in food. The last work programme 2018 - 2020 of Societal Challenge 2 (Food Security, Sustainable Agriculture and Forestry, Marine, Maritime and Inland Water Research and the Bioeconomy) in Horizon 2020, the EU Framework

Programme for Research and Innovation, follows a strategic approach to address these challenges. In the call sustainable food security a pathway has been developed from functional ecosystems to healthy food, setting healthier plants high on the agenda. Moreover under the umbrella of the European Innovation Partnership for Agricultural Productivity and Sustainability (EIP-AGRI), a number of new instruments are being used to drive the concept of interactive innovation, to bridge research and practice and foster the uptake of innovations.

## Microbiome under FOOD 2030 strategy

Anistoroaei R.\*

*\*European Commission, DG Research & Innovation*

**Abstract:** The recent past has brought us obesity, malnutrition, emergence of food borne hazards, greenhouse gases, waste, and resource depletion. A radical rethink of how we produce, obtain and prepare our food, our nutritional wellbeing, and our water and energy usage is needed.

Today, complete genomes can be sequenced in a matter of days due to the increasingly rapid technological advances that have taken place. These ground-breaking research and innovation advances have opened an entirely new chapter in genomics and have also enabled the genomic characterization of other species or systems such as microbial ecosystems. Understanding what microbiomes do, what they are, and how they interact is a new scientific frontier made easier by rapid advances in genomic mapping, robotics, and chemical analysis. What we know and understand so far is that the microbiome has an essential impact on our health and food we produced, on plants and animals and on ecosystems in general. Unravelling their complexity offers huge potential for innovation and will be a major game changer in the way we manage our planet, obtain our food, make healthy plants and animals and implicitly healthy humans.

In a similar vein the “food system” approach advocated and described under the FOOD 2030<sup>2</sup> initiative would benefit hugely from knowledge of the plant, marine, animal, environmental and human microbiomes. The impact will be in strengthening and improving the overall sustainability and innovation capacity of food systems and in bringing to market new and cost-effective commercial applications. Such an approach will be in full support of the Sustainable Development Goals and climate change commitments.

## 2017-2022 Euphresco strategic research agenda

Giovani B.\*

*\*European and Mediterranean Plant Protection Organization, Paris, France*

**Abstract:** The Euphresco network for phytosanitary research coordination and funding has recently published a strategic research agenda, that sets the plant health research priorities to be addressed over the next 5-10 years. The document was developed taking into account the national research agendas of Euphresco members (organisations from more than 50 countries worldwide) and suggestions gathered from plant health experts who participated in various EPPO Panels meetings.

The document will be used by Euphresco members to organise their national and transnational research activities but will also set a common ground to increase coordination and co-operation between nationally-based, regional and international phytosanitary research programmes. The agenda's core is made of 7 research priorities (and 18 research objectives), that summarise common strategic areas to guide future phytosanitary research. Some of the research priorities will be presented, with a particular focus on those requiring multi-disciplinary collaboration, and the views of Euphresco on how to increase synergies on research activities will be discussed.

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<sup>2</sup> <https://publications.europa.eu/en/publication-detail/-/publication/709af455-c03d-11e6-a6db-01aa75ed71a1>



# Poster Sessions

## Session 1 - *Xylella fastidiosa*: a global threat

### 1.1 Absence of *Xylella fastidiosa* in Jordan: Preliminary Survey Results

Al-Karablieh N.\*

\*University/Public Research Institute, Hamdi Mango Center for Scientific Research| The University of Jordan, Amman (JO)

**Abstract:** *X. fastidiosa* is a global plant pathogen that threat most plant crops. Its recent spread in Europe has ringed the alert alarm in whole Mediterranean region. The current project is conducted to plan strong strategies for restriction the entrance of *X. fastidiosa* to Jordan, and ensure absence of *X. fastidiosa* in Jordan. This project started with survey orchards for source of infection in potential crops; investigate host range, disease vectors, and detection of *X. fastidiosa* in the imported and locally produced plant materials in phytosanitary program. Samples from host plants were collected in the period of February 2016 to March 2017 from Amman, Al-Mafraq and Irbid; olive (22 samples), grapevine (21), stone fruits (37), pome fruits (15), oleander (4), rosemary (4) ornamentals plants (4), Walnut (2), Guava (2), xylem-feeding insects (10), beside different plant materials imported to Jordan (46). These samples were analyzed by isolation on BCYE medium, serological techniques (ELISA) and by conventional PCR using RST31 and RST33 primers according to EPPO, 2016. The results of PCR amplification form the developed colonies on BCYE, the Elisa assay on plant saps and PCR amplification form gDNA extracted from plant materials showed negative results in comparison to the positive control used. These results preliminary indicate absence of *X. fastidiosa* in Jordan. Furthermore, more scale and periodic survey will be conducted to ensure its absence in Jordan.

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Loconsole G, Potere O, Boscia D *et al.* 2014b. Detection of *Xylella fastidiosa* in olive trees by molecular and serological methods. J Plant Pathol 96:7–14.

### 1.2 *Xylella fastidiosa* preliminary results for Albania

Cara M.\*, Mercuri J., Koka E., Bacaj M., Cara O.

\*University/Public Research Institute, Agricultural University of Tirana, Universiteti Bujqesor i Tiranës, Tirane, (AL)

**Abstract:** *X. fastidiosa* has been reported as responsible for a devastating disease on olive trees in Apulia region (South–Eastern in Italy) characterized by a quick decline syndrome. The identification of *X. fastidiosa* in the 'olive quick decline syndrome' affected trees represents the first confirmed detection of this bacterium in the European Union. Since, *X. fastidiosa* is a regulated quarantine pathogen and Albania is a neighbour country with Italy, the risk of introduction and spread of this bacterium is very high. Since 2014, technicians and farmers reported the presence of similar symptoms associated with leaf scorch and dieback of olive tree branches in Lushnja, Vlora and Saranda regions. To assess the occurrence and distribution of the pathogen in Albania, samples of twigs from olive cultivars imported from Italy or local varieties and oleander were analyzed by serological techniques (ELISA, DTBIA) using *X. fastidiosa* – specific antibodies and PCR using three set of primers. Results demonstrated that all olive and oleander collected samples were free from the pathogen. Nevertheless, continuous monitoring and rigorous control measures of propagative materials are necessary to prevent the introduction of *Xylella fastidiosa* in Albania. Moreover from 21.08.2014 has banned by Order No. 409 of the Minister of Agriculture, the import of olive seedlings from Apulia region.

## Bibliography

Saponari M., D. Boscia, F. Nigro & G.P. Martelli, 2013. Identification of DNA sequences related to *Xylella fastidiosa* in oleander, almond and olive trees exhibiting leaf scorch symptoms in Apulia (Southern Italy). *Journal of Plant Pathology* 95,659–668.

### 1.3 Australia's approach to managing the risk of *Xylella fastidiosa*

Davis K.C. & Dall D.J.\*

\*National Authority, Department of Agriculture & Water Resources, Canberra, (AU)

**Abstract:** *X. fastidiosa* is a pest of major concern to Australia. In order to maintain its current *Xylella*-free status, Australia introduced emergency measures in November 2015 (further amended in January 2016), requiring imports of nursery stock (cuttings, rooted plants, budwood and some corms and bulbs) and plant tissue culture material of 89 known *Xylella* host plant families to be tested and certified (off-shore or on-shore) before being allowed unrestricted entry. The emergency measures, which remain in force as of April 2017, are in addition to a range of standard national biosecurity measures, and are further supplemented by *Xylella*-focussed activities that include development of a full pest risk assessment, initiation of surveillance activities, preparation of an incursion contingency plan, maintenance of diagnostic capabilities, and international engagement with researchers and relevant research activities. This contribution will summarise the current position of Australia's activities in managing this biosecurity risk.

## Bibliography

<http://www.agriculture.gov.au/import/goods/plant-products/how-to-import-plants/xylella/notification-amended-emergency-quarantine-measures#3>

### 1.4 Potential vectors of *Xylella fastidiosa* and their host plants found in Germany

Markheiser A.\* and Maixner M.

\*JKI, Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Fruit Crops and Viticulture Geilweilerhof, Siebeldingen, (DE)

**Abstract:** Xylem feeding Auchenorrhyncha insert their stylets deep into plant tissues to feed on xylem-sap which may be colonized by the bacterium *X. fastidiosa*. The pathogen can lead in susceptible plants to symptoms like yellowing, leaf scorch, withering of branches and finally dieback. The Meadow Spittlebug (*Philaenus spumarius*) is the only vector of the pathogen identified in Europe so far, but the potential role of other xylem feeders needs to be investigated. In the course of the European XFACTORS project, surveys are carried out to identify the prevalent xylem-feeding species (Hemiptera: Cicadidae, Cercopidae, Aphrophoridae and Cicadellinae) in susceptible crops and their natural host plants in Germany. A preliminary list of xylem feeding Auchenorrhyncha and their principal host plants identified so far is presented based on the results of the first season of the project activities.

#### Acknowledgment

This work has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N. 727987 "Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy XF-ACTORS".

### 1.5 Preventing *Xylella fastidiosa* introduction in Serbia - challenges in pathogen detection

Obračović A.\*, Zlatković N., Prokić A., Pavlović Ž., Ivanović M.

\*University of Belgrade, Belgrade – Zemun (RS)

**Abstract:** Recent interceptions of *X. fastidiosa* in asymptomatic ornamental plants imported from Central America into Europe imposed restrictions in international trade of live plant material. Serbia is considered a *Xylella*-free country and the status has been checked periodically by surveying and sampling of potential host plants mainly of the external origin. Since the establishment of *X. fastidiosa* in Italy and later in France, a number of plant species inspected and sampled for the analysis at the border crossings have increased rapidly. Received plant samples were tested first by conventional PCR according to Minsavage *et al.* (1994). DNA was 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 was used as a positive control. No pathogen was detected in 173 samples in 2016 and 126 samples in 2017. However, a few reactions associated with testing of sweet cherry samples repeatedly produced a faint band of similar size as the expected product (ca. 700 bp). The samples were additionally tested by qPCR (Harper *et al.* 2010, erratum 2013). The qPCR results were negative for the presence of the targeting gene (rim M). Consequently, the samples were considered pathogen free. The conventional PCR false positive signal confirmed the necessity for continuous improvement of the practice and expertise facilitated by the POnTE project (GA 635646).

#### Acknowledgment

This work has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N. 635646 "Pest Organisms Threatening Europe POnTE".

#### Bibliography

Minsavage *et al.* 1994; Harper *et al.* 2010, erratum 2013.

## 1.6 Pest OrgaNisms Threatening Europe

The Horizon 2020 Project –PonTE Consortium\*

*\*CNR IPSP - Institute for Sustainable Plant Protection, Bari (IT)*

**Abstract:** POnTE (Pest OrgaNisms Threatening Europe) is a four-year research project started on November 1, 2015 and funded by the EU Horizon 2020 programme (Grant Agreement Number 635646). It focuses on minimising the risk of the introduction/impact of emerging pests threatening EU agriculture and forestry. The targets are: 1) *X. fastidiosa* and its vectors in olive, grapevine, citrus, stone fruit, ornamentals and landscape trees of high socio-economic importance; 2) '*Candidatus Liberibacter solanacearum*' and its vectors affecting a number of strategic crops such as potato, tomato and carrot; and 3) *Hymenoscyphus fraxineus* and *Phytophthora* spp., fungal pathogens seriously affecting broadleaf and conifer species in forest ecosystems. The proposal fosters and promotes a multi-actor approach and transnational research collaborations among 25 Partners at the forefront of research in plant protection, agro-engineering and economics. It involves key industries/SMEs that develop diagnostic kits and services, agrochemical and seed companies as well as stakeholder groups. Targeted pests, their vectors and the host response are explored using innovative approaches (e.g. NGS, transcriptomics). Disease surveillance and epidemiology addressed by current methods are integrated with improved survey protocols and remote sensing. The innovative IPM includes studies of the microbiome to develop sustainable solutions in line with the EU plant health legislation. New knowledge gained within the POnTE project will result in an outcome-based pest prevention and management work plan. End-users are participating in the development of the project for the implementation of the practical solutions derived from the research outcomes to solve these serious emerging diseases.

More information and updates on project activities are available on the POnTE website and social media channels:

Website:	<a href="https://www.ponteproject.eu/">https://www.ponteproject.eu/</a>
Twitter account:	<a href="https://twitter.com/ponteprojecteu">https://twitter.com/ponteprojecteu</a>
Facebook page:	<a href="https://www.facebook.com/ponteprojecteu">https://www.facebook.com/ponteprojecteu</a>

## 1.7 *Xylella fastidiosa* Active Containment Through a multidisciplinary-Oriented Research Strategy

The Horizon 2020 Project - XF-ACTORS Consortium\*

*\*CNR-IPSP - Institute for Sustainable Plant Protection, Bari (IT)*

**Abstract:** "*X. fastidiosa* Active Containment Through a multidisciplinary-Oriented Research Strategy" (XF-ACTORS) is a four-year Project started on November 1st, 2016 and funded by the Horizon 2020 program (Grant Agreement Number 727987). It aims to establish a multidisciplinary research program to answer the urgent need to improve prevention, early detection and control of *X. fastidiosa*.

Currently, *X. fastidiosa* has been detected into Italy, where it is causing severe damage to olive crops in the Apulia region, in France, where so far it is limited to ornamental plants and some landscape trees in Corse and in the PACA region, and in Spain, where the bacterium has been detected in the Balearic Islands and, recently, in mainland Spain in the province of Alicante. In Spain the situation appears to be complex, with the occurrence of strains belonging to different subspecies, including the subsp. *fastidiosa* known to be associated to the Pierce's disease.

The overall goal of the research program is to develop scientific knowledge on the pathogenicity, transmissibility and host susceptibility to the *X. fastidiosa* strains recovered in the EU outbreaks, toward the implementation of tools for pest risk assessment, for prevention and reduction of the impact of the *Xylella*-induced diseases. The research actions are complementary to those carried out under the Project POnTE, thus ensuring effective cooperation and continuity with currently ongoing efforts.

Critical information on the pathogen biology, epidemiological traits and hosts under threat have been developed during the first year of the project, with the valuable guidance of the American research groups with long-established research. Similarly, research on the vector(s) are disclosing some preliminary information that will support the development of effective and sustainable vector control strategies.

The project workplan includes significant research investigations for the identification of olive genetic resources resistant to the bacterial infections, as well as testing the effectiveness of selected bio-molecules and antagonists for the control of the bacterial infections in plants.

Early detection of the infections represents a pillar task of the project, and promising results have been gathered by combining remote sensing approach with inspections and measurements at field and tree level, integrated with innovative tools for on-site detection.

More information and updates on project activities are available on XF-ACTORS website and social media channels:

Website:	<a href="http://www.xfactorsproject.eu/">www.xfactorsproject.eu/</a>
Twitter account:	<a href="https://twitter.com/xf_actors">www.twitter.com/xf_actors</a>
Facebook page:	<a href="https://www.facebook.com/xf.actors.project/">www.facebook.com/xf.actors.project/</a>

## Session 2 - *Xylella fastidiosa*: biology and genetics

### 2.1 Geographic Distribution and Genetic Diversity of *Xylella fastidiosa* in Mexican Vineyards.

Aguilar-Granados A.\*, Kameyama-Kawabe L., Hinojosa-Moya J., Ruiz-Medrano R., del Carmen Montes-Horcasitas M., Xoconostle-Cázares B.

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**Abstract:** *X. fastidiosa* is a phytopathogenic bacterium living in xylem and is present in many plants. Mexico is an important producer of vines, and is necessary to know *Xylella* distribution and genetic variability, as complementary information for the management of the disease. A total of 95 samples were taken in the main wine producing areas (Baja California, Coahuila, Querétaro States) and analyzed in the bacteriology laboratory of the National Center of Phytosanitary Reference. The detection of the bacteria was performed using qPCR, and the strains were characterized by multi-locus

sequence analysis (MLSA), and comparing to the sequences type (ST) of the corresponding subspecies with the database (<https://pubmlst.org/xfastidiosa/>). The MLSA identified the vine isolates in the States of Baja California, Coahuila and Querétaro, corresponded to *X. fastidiosa* subsp. *fastidiosa*. The presence of the different subspecies in the clades shows that the isolates of the north of the country bear identity with the sequences type ST1, ST3 and TS4, which are similar to those present in California (USA). However, the isolates from Querétaro present an unreported genetic variability, although with greater homology to the isolates present in Costa Rica (isolates of vine and coffee). It is necessary to correlate the variability observed with its possible specificity by another range of hosts and virulence, as well as their presence in other vector insects.

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## 2.2 *Xylella fastidiosa*, research performed in a Dutch project

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**Abstract:** The risk of introduction of the A1 quarantine bacterium *X. fastidiosa* in the Netherlands, where host plants of the bacterium are imported from countries, where the pathogen is endemic, is estimated as high. Plants can be infected asymptotically. During testing, infections may be missed due to a low density of the pathogen, the low infection frequency and/or the uneven distribution in the host. As a xylem-invader, the pathogen will easily survive external decontamination of plant material and transport. *Philaeus spumarius*, the vector found to transmit *X. fastidiosa* is also present in the Netherlands, presenting a risk on dissemination after introduction. A public – private financed research project started on *X. fastidiosa* in the Netherlands focusing 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.
- Development of improved multilocus TaqMan assays with extraction and amplification controls.
- Exploration of the possibilities of NGS for rapid detection and characterization of the pathogen in plant material.
- Studies on plant colonization, dissemination and survival. Host plants studies will be performed on various trees and ornamentals relevant for the Dutch sector.

Within the Euphresco network we will focus on several aspects of the bacterium relevant for horticulture and arboriculture in the Netherlands. Preliminary results are presented.

## 2.3 Is *Xylella fastidiosa* a risk for Belgium? Ongoing investigations on potential hosts and insect vectors

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**Abstract:** Based on the previous EFSA opinion on the risks to plant health posed by *X. fastidiosa* in the EU territory, potential hosts plants for *X. fastidiosa* (EFSA, 2014), like *Prunus domestica* cv Opal, *Quercus petraea* and *Salix alba* have been selected and inoculated with different strains of *X. fastidiosa* subsp. *fastidiosa* and *multiplex* as well as the CoDiRo strain, to evaluate their susceptibility. The potential for the bacteria acquisition through artificial diets (Killiny and Almeida, 2014) and artificially infected plants with a GFP tagged strain (Newman et al., 2003) is also studied for potential



insect vectors like *Cercopis vulnerata*, *Cicadella viridis*, *Philaenus spumarius*, *Aphrophora alni* and *Aphrophora salicina*. With the view of supporting field surveys, a PCR detection scheme combining the detection of both the bacteria and potential insect vectors has also been developed. Such key elements like bacteria's host range or potential insect vectors are discussed in relationship with the risk for Belgium.

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## 2.4 Insights into *X. fastidiosa* virulence and host specificity revealed by multi-omics approach

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**Abstract:** Several determinants have been implicated in the *X. fastidiosa* pathogenicity and virulence, most of them discovered through studies with 9a5c and Temecula-1 strains, causal agents of CVC and PD, respectively (Chatterjee et al. 2008). Studies of other *X. fastidiosa* strains isolated from distinct plant hosts revealed genotypic differences that correlate to the distinct phenotypes displayed by these strains (Coletta et al 2017, Jacques et al 2016, Jacques et al. 2016, Marcelletti and Scortichini 2016, Zhang et al. 2015). We have collected genomic, transcriptomic and proteomic data for selected *X. fastidiosa* strains aiming to expand the knowledge about their determinants of host specificity, host colonization and virulence. Comparative genome analyses revealed the diversity of mobile genetic elements-related regions, which are more abundant in South American than in the North American strains. RNA-seq analysis of selected strains cultivated in nutrient-rich or in nutrient-poor media showed that a nutrient stress condition enforces rapid changes in *X. fastidiosa* gene expression during growth. Transcripts related to type-IV pili and proteases were more abundant in early growth phase while transcripts encoding cell wall degrading enzymes prevail in late growth phase. Our results also suggest that gene expression signatures seem to more dependent on genotype than on medium type or growth phase. These genomic and transcriptomic datasets are being integrated to shotgun proteomics data to unveil novel host specificity, host colonization and virulence determinants. Supported by FAPESP, CNPq and CAPES.

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## 2.5 Characterization of *Nicotiana tabacum* as an alternative host for *X. fastidiosa* subsp. *pauca*

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**Abstract:** The long latency period of *X. fastidiosa* for the majority of perennial hosts, such as citrus and olive, makes difficult the advance of plant-pathogen studies. Therefore the use of susceptible model plants is required. In this work we show the results of infection and symptoms of the tobacco varieties Havana and RP1, inoculated with three Sequence Types (ST) of *X. fastidiosa* subsp. *pauca* (ST13, ST16, and ST53). Eighty days after inoculation the percentage of infected plants was 100% in Havana and RP1 inoculated with ST13 (9a5c citrus-strain). With the ST16 (MF01- olive strain from Brazil), the percentage was 83% in Havana and 100 % in RP1. The lowest percentage of infection was observed with ST53 (CoDiRO strain) with 55 and 78% for Havana and RP1, respectively. Symptoms of small dark lesions surrounded by yellowing at margins were observed only for the ST13 colonizing either Havana or RP1. Significant reduction in plant size and development were observed for all strains infecting both varieties, but more significantly for RP1. In addition, leaf-yellow blotches between secondary veins were mainly observed on RP1 but with gradual intensity (more to less) when the plants were infected with the ST13, ST16 and ST53, respectively. These results demonstrated that tobacco is a good model plant for *X. fastidiosa* subsp. *pauca*, although different varieties could show different level of infection and symptoms. In this work we verified that both varieties were good hosts for ST13, but RP1 was better for ST16 and ST53.

### Acknowledgment

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## 2.6 Metabolomic characterization of xylem sap of different olive cultivars growing in Spain

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**Abstract:** With the goal of understanding the nutritional requirements of xylem-limited microbiome, the chemical composition of xylem sap extracted from two *Olea europaea* var. *europaea* cultivars (Picual and Arbequina) was evaluated. Selected plants were established on their own feet or grafted onto rootstocks of *Olea europaea* var. *sylvestris*. Xylem sap of olives branches was collected using a external 45-cm external Scholander Super Chamber. Metabolome analysis was performed by proton nuclear magnetic resonance (NMR) spectroscopy-based study and the ionome analysis was performed by inductively coupled plasma with optical emission spectroscopy (ICP-OES). In total 26 metabolites were identified by NMR spectroscopy, including amino acids (alanine, arginine, aspartate, glutamate, glutamine, isoleucine, leucine, methionine, proline, threonine, tyrosine, and valine), sugars (glucose, fructose, mannitol, myo-inositol, and sucrose), organic acids (formic, fumaric and succinic acid),

alcohols (ethanol and methanol), and other molecules. On the other hand ICP-OES allowed the quantification of 14 elements and five inorganic anions. Xylem sap analysis will lead to a better understanding of the biology and complex nutritional requirements of olive xylem-inhabiting microorganisms, including *Xylella fastidiosa*, and to help designing artificial growing media to improve culturing of the olive microbiome. Study supported by Projects 727987 XF-ACTORS (EU-H2020) & AGL2016-75606-R (MEIC Spain and FEDER-EU)

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## 2.7 *X. fastidiosa* associated with extensive mortality of almond trees in Mallorca

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**Abstract:** In 2008 a new disease causing mortality of almond trees appeared in orchards located in Son Carrió, east of Mallorca. Disease symptoms were described 'as rapid collapse of branches during mid-summer, chlorosis of leaves, which suddenly wilted, as well as bud and shoot'. In the following two summers the disease had spread at a rate of mortality never seen before in Majorca, and five years later thousands of almond trees had died throughout the island. Eventually, the disease was associated with a complex of pathogenic fungi of the genera *Phaeoamoniella*, *Phaeocremonium* and *Botryosphaeria*, although the causal agent was not determined (Gramaje *et al.* 2012).

In October 2016, *X. fastidiosa* was officially detected in a Garden Centre in Porto Cristo, 1.5 kms from the first focus of the almond disease. Multilocus-sequence typing of three isolates from infected *Prunus avium* identified them as *X. fastidiosa* subsp. *fastidiosa*, ST1, the same genotype group causing Almond leaf scorch disease (ALSD) in California. To date 46 positives from Almond have been reported.

Here we will show the parallelism of the outbreak of the Olive Quick Decline Syndrome caused by *X. fastidiosa* in Apulia, Italy, with that of the ALS in Majorca. We will provide evidence reviewing the etiology; the epidemiology; the genotypes of the pathogen associated with symptomatic almond trees, and we will present the results of ongoing research to revisit what might have been the first outbreak of *X. fastidiosa* in Europe.

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## 2.8 Pathogenicity of some fungal species associated with olive quick decline syndrome

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**Abstract:** Several fungal species have been found associated with the Olive Quick Decline Syndrome (OQDS) caused by *X. fastidiosa* CoDiRO strain, among which some have long been recognized as primary pathogens of many plants (i.e. *Phaeocremonium* and *Pleurostomophora* spp.), whereas other have not been described before. The new species *Ps. oleae* and *Ps. oleicola*, were associated with brown wood streaking of various olive varieties, both on young and centenarian trees. In order to ascertain the role of these fungi in the OQDS, pathogenicity tests were performed both in glasshouse, on young plants, and in the open field, on centenarian olive plants cv Cellina di Nardò. In glasshouse



tests, fungal strains (*Ph. aleophilum* B1a, *Ph. rubrigenum* N20, *Ps. oleae* Fv84, *Ps. oleicola* M24, *Pseudophaeomoniella* sp. M51), were inoculated alone or in combination with *X. fastidiosa* CoDiRO strain. For the test in the field, naturally *X. fastidiosa* CoDiRO-infected and uninfected centenarian plants were inoculated with the same fungal strains. Results from the glasshouse tests, collected two years after the inoculation, indicate that fungal strain(s) alone are able to colonize the olive xylem, and to cause wood streaking. However, only few twigs showed wilting symptoms. Conversely, the combined inoculations with *X. fastidiosa* CoDiRO, induced extensive OQDS wilting symptoms. To date, results from the field observation 8 months after fungal inoculation show no differences in symptoms severity between *X. fastidiosa* infected and healthy plants.

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## 2.9 Role of argininosuccinate synthase in Pierce's Disease development in grapevines

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**Abstract:** Pierce's disease (PD) in grapevines is among several deadly plant diseases caused by strains of *X. fastidiosa*. The analysis of the secretome of wild type *X. fastidiosa* Temecula1 (WT) revealed LesA (lipase/esterase) and PrtA (protease) as the most abundant secreted proteins involved in virulence, cell growth, motility, and biofilm formation. Enzymatic activity of LesA has also been shown to be closely associated with PD symptoms. *X. fastidiosa* mutants deficient in prtA display an obligate planktonic phenotype, secrete high levels of LesA, are hypervirulent and accumulate high levels of argininosuccinate synthase (ArgG). ArgG is an enzyme involved in arginine biosynthesis and was previously characterized as virulence factor in the rice pathogen *Xanthomonas oryzae* pv *oryzae*. High levels of arginine in infected leaves of citrus was also associated with citrus variegated chlorosis caused by *X. fastidiosa*. Therefore, we aim to investigate if the arginine metabolism that appears to be closely associated with LesA activity contributes to *X. fastidiosa* growth and virulence. Thus, argG coding sequence in *X. fastidiosa* WT (PD0291) was disrupted and grapevines inoculated with *X. fastidiosa* argG- mutant presented less symptomatic necrotic regions on the edges of the leaves compared with *X. fastidiosa* WT after 15-weeks inoculation. The relationship of arginine metabolism with PD symptom development and the expression of LesA will allow a better understanding of how arginine contributes to *X. fastidiosa* virulence in planktonic phase leading to disease development.

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## 2.10 Lipidome analysis of *X. fastidiosa* strain CoDiRO

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**Abstract:** In the frame of the Xf-actors project, we focus attention on signals involved in plant-pathogen interaction to develop innovative and sustainable approaches to control *Xylella fastidiosa*, associated with the olive quick decline syndrome.

Lipids, components of the plasma and intracellular membranes, provide different biological functions. During plants-pathogen interactions lipids have different roles, as pathogen perception, signal transduction and downstream defence responses. Different bacterial species display diverse membrane compositions and even the membrane composition of cells is not constant but depends on the environmental conditions. Oxidized fatty acids are an important class of signalling molecule especially related to stress responses. Recently, other authors reported that oxylipins have a regulation activity in motility, biofilm formation and virulence in *Pseudomonas aeruginosa*.

In this study, we explore the oxylipins signals of *X. fastidiosa* CoDiRO CFBP8402 in pure culture grown at 25°C. Moreover, we exploit the lipids signals during the plant-pathogen interaction with the model plant *N. tabacum* "Petite Havana SR1" after artificial inoculation with CoDiRO. The analysis were performed by LC-MS/MS in dynamic MRM modality allowing quantification of oleic, linoleic and linolenic acid -derived oxylipins. The results showed the presence of oxidized fatty acids in *X. fastidiosa* CoDiRO strain in pure culture and in inoculated tobacco plants.

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## 2.11 Identifying *Xylella fastidiosa* host adaptation candidate genes: the case of *X. fastidiosa* subsp. *pauca* isolates and olive trees in Italy

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**Abstract:** The emergence of a *X. fastidiosa* subsp. *pauca* genotype in southern Italy is causing a devastating disease in olives trees. Genomic analyses have shown that isolates collected from olive trees in Apulia belong to the same sequence type ST53, together with coffee and oleander isolates from Costa Rica. This case represents an opportunity to study a relatively recent adaptation of *X. fastidiosa* to a new host. Forty isolates have been collected from olives trees in Apulia and their complete genomes sequenced. The analysis of these sequences should enable us to identify candidate genes for *X. fastidiosa* host adaptation.

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## 2.12 Epidemiology and management of almond leaf scorch disease in the San Joaquin Valley of California, USA

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**Abstract:** Almond leaf scorch disease has been a chronic problem in the San Joaquin Valley of California for >50 years. The green sharpshooter, *Draeculocephala minerva*, is the most abundant vector of *X. fastidiosa* in and around almond orchards and nurseries throughout the San Joaquin Valley. The green sharpshooter prefers grasses over almond foliage. Accordingly, movement of green sharpshooter into almond orchards is transient, with risk of vector movement into almond orchards and nursery plantings increasing based on proximity to known source habitats (irrigated pastures and weedy alfalfa fields). Because the vector does not reside in orchards, infections are due to primary spread with little secondary spread. Yield of chronically infected trees was reduced by 20 to 40% compared to healthy trees for the cultivars 'Nonpareil' and 'Sonora'. Surveys conducted in commercial almond orchards determined that almond leaf scorch disease was widespread, but that incidence was typically low (<1%). Incidence varied with cultivar, suggesting differences in scion susceptibility. Disease severity was also affected by rootstock. Evaluation of almond rootstocks under development as part of the USDA-ARS almond rootstock improvement program determined that *X. fastidiosa* reaches high population densities in some, but not all rootstock lineages. Effects of four rootstocks ('Nemaguard', 'Okinawa', 'Nonpareil', and Y119) on disease severity in the susceptible scion 'Sonora' was evaluated in a 5 year field study. Artificial inoculations of 'Sonora' grafted on the rootstocks 'Okinawa', 'Nonpareil', and Y119 resulted in chronic infections. However, artificial inoculation of 'Sonora' grafted on the rootstock 'Nemaguard' resulted in remission of leaf scorching symptoms, with *X. fastidiosa* undetectable after two years. Collectively, the results indicate that a *X. fastidiosa*-resistant trait in the rootstock can be valuable for maintaining low incidence of disease and that understanding the degree of susceptibility to *X. fastidiosa* in complex hybrids should be an important part of rootstock development.

## 2.13 Characterization of *X. fastidiosa* from different hosts in Argentina

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**Abstract:** In Argentina, *X. fastidiosa* infects different crops of agricultural importance such as almond (northwest), citrus (northeast) and recently it has been detected in olive in production areas of Córdoba, La Rioja, Catamarca and Buenos Aires provinces. Regarding this last host, the bacterium was first detected in December of 2013, in olive orchards of more than 50 years old, mainly cv. Arauco, located in Aimogasta (La Rioja) and Cruz del Eje (Córdoba), where plants were observed expressing a marked decline and symptoms such as some branches displayed desiccated leaves at the top and basal leaves with apical scorching. The cv. Arauco, that is only present in South America, is the most cultivated and appreciated one due to its attributes of high production and double purpose, although it has the highest number of plants infected by the bacteria. The different strains were characterized through the Multilocus sequence typing system (MLST). The citrus and olive strains were found to belong to the sequence typing 69 (ST69) while a new ST (ST78) was identified in almond trees, both corresponding to the subspecies *pauca*. *X. fastidiosa* was not detected in citrus from the northwest region, so the presence of olive ST69 constitutes a potential danger for the citrus orchards of that region. Studies are underway to determine if the new almond strain could be transmitted to citrus and olive trees.

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## Session 3 - *Xylella fastidiosa*: host plants and resistance

### 3.1 Detection of *X. fastidiosa* in susceptible and resistant field olive trees

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**Abstract:** Olive quick Decline syndrome (OQDS) is a destructive disease characterized extensive branch and twig dieback, followed by death of the trees. Field surveys have unraveled the coexistence under the same cultivation conditions of olive trees showing severe or conversely very mild and scattered desiccation phenomena. Such evidences prompted investigations on the differential response of the olive cultivars to *Xylella*-infections. The first outcome of these studies indicated consistent lower bacterial population size (ca.  $10^4$  CFU/ml) associated to the infected trees showing milder and no symptoms. We tested a large number of trees belonging to highly susceptible (Cellina di Nardò and Ogliarola salentina) and resistant cultivars (Leccino), and correlated the susceptibility (presence/absence of symptoms) of the cultivars with the diagnostic results of the ELISA and qPCR tests. All 500 trees belonging to cvs Ogliarola salentina and Cellina di Nardò and showing severe symptoms tested positive for *X. fastidiosa* in qPCR, with 97.7% testing positive in ELISA. Whereas, when 100 trees of the cv Leccino, mostly symptomless, were tested by both methods, the bacterium was detected in 34,6% of the trees by qPCR and in 14,8% by ELISA. These results while support the finding of lower bacterial population size in the resistant olives, highlight that the implementation of the *Xylella*-monitoring program for olives should take into account the olive cultivar.

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### 3.2 Analysis of defense-related gene expression in citrus hybrids infected by *X. fastidiosa*

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**Abstract:** Resistance to *X. fastidiosa* was evaluated in 10 six year old hybrids of crosses between Murcott tangor [*Citrus reticulata* Blanco x *Citrus sinensis* (L.) Osbeck] and Pera sweet orange [*Citrus sinensis* (L.) Osbeck] under field conditions. Healthy hybrids were grafted with buds collected from plants of Pera sweet orange infected with *X. fastidiosa*, forming a plant with two scions. We evaluated the symptoms of citrus variegated chlorosis (CVC), bacterial multiplication and differential gene expression in both scions. Hybrid scions had no symptoms, however three of them had bacteria detected by quantitative PCR (qPCR). Indeed, all scions that originated from buds collected from plants of Pera sweet orange infected with *X. fastidiosa* showed symptoms of CVC. We next monitored 13 defense-related genes by real-time quantitative PCR (qPCR). We suggest that some of these genes are involved with resistance of the hybrids to *X. fastidiosa*, since their expression was significantly higher in the resistant hybrid scions than in tolerant hybrids and scions originated from infected Pera sweet orange buds. Furthermore, we carried out Pearson's correlation analysis between gene expression, multiplication of bacteria and the presence of symptoms. Canonical correlation analysis revealed a relationship between the expression of these genes and hybrid scions, and also between scions that originated from infected buds and the presence of the bacteria and plant symptoms.

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### 3.3 Conserved genetic defense response against *X. fastidiosa* subsp. *pauca* in olive and citrus

De Souza A.A.\*, Takita M.A., Rodrigues C.M., Coletta-Filho H.D., Giampetruzzi A., Morelli M., Saponari M., Boscia D., Saldarelli P.

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**Abstract:** *X. fastidiosa* subsp. *pauca* causes diseases in citrus and olive plants. Fortunately, there are citrus species and olive varieties more tolerant to *X. fastidiosa* and therefore good genotypes to search for genetic sources of resistance. Following this approach, global gene expression analyses were recently achieved using *Citrus reticulata* cv. Ponkan and *Olea europaea* cv. Leccino allowing the identification of potential genes involved in plant defense response. Thus, the objective of this work was to identify key genes involved in common genetic defense responses that could be further explored to get resistant varieties. Overall we identified two main mechanisms for both plant species: i. Bacteria recognition and ii. Cell wall fortification. The former involve the expression of pattern-recognition receptors, which recognize pathogen molecular patterns and trigger cell defense responses. Some of these receptors belong to the LRR-XII group which contains cell surface immune receptors. The latter involves downregulation of genes in tolerant host such as expansin, pectate lyases and polygalacturonases, related with cell wall expansion and degradation. This suggests that in tolerant hosts, plant cell recognizes *X. fastidiosa* and reprograms the cell wall development to impair its colonization through the xylem vessels. Therefore these genes represent good candidates to be explored aiming their use in breeding and/or genetic engineering program.

#### Acknowledgment

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### 3.4 Overgrafting as solution to save old olives and testing germplasm

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**Abstract:** An extensive trial, consisting of overgrafting 12,5 hectares in 3 private olive orchards (located in the infected area of Salento, in Presicce and Ugento) with 2 different levels of symptom's expression, started in April 2016 and completed in the spring 2017 without public funding, is presented. With the collaboration of germplasm collections, scientific institutions and consortia, 260 among different cvs and clones, representative of 19 olive growing countries and all Italian regions, were collected and grafted, by patch grafting, using a complete randomized scheme with 5 replicates and 3 controls on each plants (the susceptible cv Ogliarola salentina and 2 resistant Leccino and Frantoio). Starting from the observation, in the oldest heavily infected area, of almost asymptomatic plants where Leccino were grafted over 10 years ago on trunk of susceptible cvs, the experimental idea has several objectives: try to save the centenary trees by substituting the canopy with resistant varieties; define a grafting protocol adapted to the local conditions; develop a new method "Xylella Quick Tolerance Test" aimed to evaluate the susceptibility of a large quantity of germplasm in shorter time; c. identify a larger number of resistant/tolerant cvs and sources of resistance; d. estimate the potential risk of spreading of the OQD disease in other territories. Due to the good engraftment % and development of grafts the first preliminary evidences are expected in the present growing season.

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### 3.5 Search for resistance to olive quick decline syndrome caused by *X. fastidiosa*

León L.\*, Belaj A., De la Rosa R., Serrano A., Boscia D., Saponari M.

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**Abstract:** The olive quick decline syndrome (OQDS) is a novel disease recently emerged in southern Italy and currently threatening the Mediterranean olive-growing area. The disease was found to be associated with infections caused by the exotic bacterium *X. fastidiosa*, well known in the Americas as the causal agent of severe diseases of important crops, i.e. grapevine and citrus. In these species, looking for resistance is regarded as one of the most promising long term control strategies. In olives, cultivars displaying differential phenotypes have been observed in the field, with symptoms ranging from virtually no decline and limited desiccation in Leccino and FS-17® to severe decline and death cvs Cellina di Nardo and Ogliarola Salentina. However, the large variability of olive genetic resources, including both cultivated and wild olives remains to be explored. Several experiments are currently ongoing aiming at detecting new sources of resistance in plant materials representative of the genetic and geographical variability of the species, including commercial cultivars, genotypes from other *Olea europaea* subspecies, and selections from breeding programs. Identified resistant sources could serve as parental for breeding programs, as currently underway for other vascular diseases such as *Verticillium* wilt. The status and challenges of the currently ongoing activities will be presented.

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### 3.6 The endophytic microbiome of *X. fastidiosa* susceptible and resistant olives

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**Abstract:** A multi-factorial strategy is required to co-exist with *X. fastidiosa* infections, which are devastating olive trees in the southern area of Apulia (Italy). Observations in the outbreak area can provide information on potential approaches for containment. Olive cvs Leccino and FS17 show lessened symptoms and host lower bacterial populations (1,2) than cvs Ogliarola salentina, Cellina di Nardò and Kalamata. We are evaluating whether microbial communities inhabiting the xylem vessels of olive cvs showing different susceptibilities to *X. fastidiosa* -infection play a role in resistance. To explore these endophytic microbiomes, a whole-metagenome shotgun analysis is currently ongoing. *X. fastidiosa* -infected and healthy olive plants of the cultivars FS17, Leccino and Kalamata, were selected from the same plot to limit the influence of diverse soil composition and crop management. Shotgun sequencing of DNA extracted from the xylem tissues will be used to investigate the microbiome community by bio-informatic analysis. Moreover, efforts to isolate culturable microorganisms to be used in antagonistic assays against *X. fastidiosa*, will be performed. Concurrently, the *X. fastidiosa*-biocontrol potency of *Paraburkholderia phytofirmans* PsJN strain, whose beneficial effects in the reduction of symptoms in Pierce's Disease (3) have been recently described, are under evaluation. We are testing the ability of *P. phytofirmans* to colonise xylem vessels and interact with *X. fastidiosa* in tobacco and olive.

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## Session 4 - *Xylella fastidiosa*: pathogen and disease control in the host plants

### 4.1 In vitro activity of antimicrobial compounds against *X. fastidiosa* causing OQDS in Apulia (IT)

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**Abstract:** Olive quick decline syndrome (OQDS) caused by *X. fastidiosa* is currently causing severe damages to the production and reducing the life span of the plants in the Salento peninsula of Apulia (Italy). No effective means of control of *X. fastidiosa* is currently available. The objective of this study was to evaluate in vitro antimicrobial activities against *X. fastidiosa* (strain Salento-1) of different classes of compounds having diverse origins, i.e. traditional antibiotics, plant-derived natural products,

and microbial metabolites. A preliminary bioassay, performed by the agar disc diffusion method, revealed that 17 of the 31 antibiotics tested did not affect bacterial growth at a dose of 5 µg. Olive mill wastewaters (OMWs), which are known to possess a broad range of antimicrobial activity, are able to inhibit *X. fastidiosa* in vitro. Most interestingly when we analysed different OMWs derived micro, ultra and nano-filtered fractions as well as some of the single phenolic compounds that they contain, we found that the OMWs micro-filtered fraction is the most effective against the bacterium but only few phenolics are active in their pure form. Also some fungal extracts and bacteria toxins showed noteworthy inhibitory effect to strain Salento-1 growth. The possible use of some of these products for curative/preventive treating OQDS-affected or at-risk olive plants will be discussed.

#### 4.2 Bactericide activity of essential oils and milk's ions against *X. fastidiosa*

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**Abstract:** *X. fastidiosa* is a bacterium that attacks the xylem of plants, transmitted by xylem-feeding insects. In Europe, outbreaks have occurred in Italy, France and Spain.

We are currently testing several molecules that may have the potential to control three subspecies of the bacterium, namely *fastidiosa*, *multiplex* and *pauca* Salento-1. Essential oils are concentrated hydrophobic liquids containing volatile aroma compounds from plants. Previous screening tests carried in our laboratory showed that some of these essential oils could inhibit several bacterial diseases. The lactoperoxidase is a bovine milk enzyme, part of the natural biological protection system present in many excretion glands, that has the ability to yield ions with antimicrobial activity. The enzyme is tested widely against human and animal pathogens but has been less investigated against plant pathogens. The potential application of essential oils and lactoperoxidase against *X. fastidiosa* started in our laboratory recently. In vitro, the three subspecies were inhibited in presence of the lactoperoxidase yielded active ions. On the hundred essential oils previously screened, a dozen got selected to be tested in vitro, and three of them inhibited the three subspecies. The next step is now to evaluate the potential of these molecules on olive trees in the region of Lecce, Italy. Phytotoxicity tests are carried and xylem-injection methods are developed in the perspective of future in vivo trials.

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#### 4.3 Does hot water treatment against *Xylella* affect the vitality of grapevine propagation material?

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**Abstract:** Notwithstanding the pathogenicity tests on several cvs proved that grapevine is not host of *X. fastidiosa*. CoDiRO strain, since 2016 hot water treatment (according with EPPO protocol - Bulletin OEPP/EPPO Bulletin, 42(3), 490-492) was adopted as mandatory preventive measures (Commission Implementing Decision (EU) 2015/2417) for all grapevine nurseries in the infected area of Salento. Even if about 10 millions plants have been treated and commercialized without failings in 2016, several market problems occurred for the fear of viticulturists not of *Xylella* but of possible direct damages on plants. In order to prove the lack of damages a specific trial was started in 2015 comparing not treated material with 3 HWT protocols (the official one 50°C for 45' and 2 more severe 50° 1h and 55° 45') and using 6 different cvs and 5 rootstocks with 75 plants for each thesis. The preliminary results indicate the lack of direct damage in the two HWT thesis at 50° and reveal just a slight delay (about 1 week) in bud breaking. The treatment at 55° cause direct damages to the main buds and a remarkable delay in breaking of secondary buds. After the 2 recent findings of the Pierce disease's agent (*X. fastidiosa* subsp. *fastidiosa*) in Germany and Spain and considering the efficacy of heat treatment against other pathogens and parasites, those results could suggest to spread the adoption of HTW as a safe and common technique in European grapevine nursery industry.

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#### 4.4 Characterization of synthetic peptides bioactivity on *X. fastidiosa* growth profile and biofilm disaggregation

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**Abstract:** The decreasing effectiveness of conventional antimicrobial-drugs has caused serious problems due to the rapid emergence of multidrug-resistant pathogens. Since the first outbreak of *X. fastidiosa* in Europe that *X. fastidiosa* plant host's producers need the development of cheap and effective antibacterial products against *X. fastidiosa*, such as for other multidrug-resistant pathogens. As a result, there is a continuous search to overcome or control such problems, which has resulted in antimicrobial peptides being considered as an alternative to conventional drugs. *X. fastidiosa* infection

depends on the establishment of biofilms that enhance antimicrobial resistance. Indeed, pathogenicity-related proteins secreted into the environment can facilitate the processes of infection and invasion. The expression of many virulence factors, such secreted proteins depends on the aggregation state of the microorganism, which exists either in a planktonic, motile condition or associated with other cells and an extracellular matrix within a biofilm. This study revealed that antimicrobial synthetic peptides effectively control and/or inhibit totally *X. fastidiosa*. In this study were tested 5 syntetic peptides - hIF-[1-11]; Dhvar-5; CA(1-7)M(2-9); Licotoxina I and D4E1, with different bioactivity targets, resulting from total Xf growth inhibition to alteration, and disaggregation of *X. fastidiosa* biofilm. Excellent antifungal activity was revealed when used on *Phytophthora cinnamomi*.

Note: The present study was developed within the scope of the *X. fastidiosa* -Freeolive project funded by FCT (PTDC/AGR-PRO/0856/2014) aiming to characterize the level of susceptibility to *X. fastidiosa* infection of olive cultivars, extensively used in Portugal and to identify the critical factors that are correlated to it.

## Session 5 - Vectors and epidemiology

### 5.1 Host-plant preference of *Philaenus spumarius* nymphs in olive orchards of the Apulia Region of Italy

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**Abstract:** Field surveys were conducted in 2015-2016 in a total of 50 olive orchards of Apulia region, by recording the incidence of the different plant species and the presence of *Philaenus spumarius* (*Ps*) nymphs on at least 100 plants within each genus (Cornara et al. 2017). Percentage of infested plants, number of infested plants over total infested plants within each orchard were determined and host-plant preference was estimated by Chesson's index (Chesson J. 1978). Nymphs were recorded on plant species of ca. 70 different Genera, with the highest rates of infestation recorded on plants belonging to the Asteraceae and Fabaceae. *Sonchus*, besides being a very common weed in the olive orchards of the region, was one of the preferred host-plants, representing up to 22% of total infested plants. On the contrary, other common weeds, such as *Papaver*, *Fumaria*, *Lamium*, *Oxalis*, *Mercurialis* and *Capsella*, were not found infested by the nymphs of *Ps*. Indeed, very rarely spittlebugs were found on plants of Cruciferae and Gramineae. In conclusion, our surveys confirmed that *Ps* has a very high polyphagy and capacity to adapt to very different plant communities. Nevertheless, densities of this insect showed great variability between orchards characterized by different plant communities, type of soil, environmental conditions and management.

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Chesson, J. 1978. Measuring Preference in Selective Predation. Ecology 59, 211–215.

### 5.2 Development of mating disruption methods to suppress populations of insect vectors of *X. fastidiosa*

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**Abstract:** The glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae), is a vector of *X. fastidiosa*, an important bacterial pathogen of several crops in the Americas and Europe. Mating communication of this and many other cicadellid pests involves the exchange of substrate-borne vibrational signals. Exploitation of vibrational signals to interfere with *H. vitripennis* communication and suppress populations could prove to be a useful tool, but knowledge of the mating behavior was insufficient to initiate development of control methods. In this study, six different *H. vitripennis* communication signals were identified and described, candidate disruptive signals (natural and synthetic) were designed and tested in the laboratory via playback to individuals and male-female pairs, and efficacy of candidate signals in disrupting *H. vitripennis* mating were validated under field conditions via playback of signals through wires used in vineyard trellis. Data support application of vibrational mating disruption as a novel method to control *H. vitripennis* populations.

### 5.3 Evaluation of sampling methods for sharpshooters and spittlebugs in Brazilian olive orchards

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**Abstract:** Sharpshooters (Hemiptera: Cicadellidae, Cicadellinae) and spittlebugs (Hemiptera: Cercopoidea) are potential vectors of *X. fastidiosa*, which is associated with Olive leaf scorch (OLS) in Brazil. Identification of key vector species involved in OLS spread depends on systematic surveys of these insects in olive orchards. Here we compared methods for sampling sharpshooters and spittlebugs in orchards of two localities in the states of Minas Gerais (Maria da Fé) and São Paulo (Cabreúva), from June/2015 to Nov/2016. In each orchard, yellow sticky cards (30 x 10 cm) were fortnightly hanged on nine olive trees. Two cards were placed per tree, at 0.8 and 1.6 m above ground. In addition, sweep net samplings were performed every 3 months on the tree canopy (2 sweeps per tree; 60 trees per orchard) and ground cover (3 samples per orchard; 30 sweeps per sample). Among 28 prevalent species of sharpshooters and spittlebugs, few were common to both areas (25%) or to both sampling methods (sweep net and sticky traps) in the two areas (26.7-50%). The prevalent species were trapped in similar numbers at 0.8 and 1.6 m by sticky cards, except for 5 species that were more frequent at 0.8 m. In contrast, few species (7.7-20%) were common by sweeping the canopy vs. ground cover. The observed variations indicate that vector surveys should be done in various localities over a growing region, by combining sticky traps (preferably at 0.8 m above ground) and sweep net (on trees and ground cover).

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### 5.4 Vector acquisition efficiency of sequence types of *X. fastidiosa* subsp. *pauca* from citrus

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**Abstract:** Different sequence types (STs) of *X. fastidiosa* subsp. *pauca* have been described in citrus causing Citrus variegated chlorosis, but little is known about their interactions with insect vectors. In this study, we compared acquisition efficiency of four STs (11, 13, 64 and 65) by two sharpshooter



vectors, *Bucephalogonia xanthophis* and *Macugonalia leucomelas*. Around 20-30 laboratory-reared (non-bacteriiferous) adults of each vector species were submitted to a 6-h acquisition access period (AAP) on parafilm membrane sachets containing cell suspensions (OD = 0.4 nm) of each ST in artificial diet (L-glutamine, L-asparagine and sodium citrate). Similar numbers of insects fed on diet without bacterial suspensions were used as negative control. After the AAP, the insects were fed for 3 days on *Catharanthus roseus* for gut washing, and subsequently tested for the presence of *X. fastidiosa* by real-time PCR. The acquisition rates of the four STs by *B. xanthophis*, measured by the percentage of PCR-positive insects, varied from 20 to 47%, but did not differ statistically. For *M. leucomelas*, ST11 was acquired at a higher rate (66.5%) than ST64 (15%), but was not statistically different from STs 13 (44.8%) and 65 (43.3%). By comparing vector species, *M. leucomelas* acquired STs 11 and 13 more efficiently than *B. xanthophis*. The variations in acquisition efficiency suggest differential ability of STs to attach and/or form biofilm in the foregut of some sharpshooter vectors.

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### 5.5 Polymorphism of potential insect vectors of *X. fastidiosa* in Majorca, Balearic Islands

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**Abstract:** The bacteria *X. fastidiosa* is a xylem-limited pathogen that can infect numerous plant species. In October 2016, *X. fastidiosa* was recorded for the first time in Majorca (Balearic Islands, Spain). The bacteria is transmitted by xylem-feeding insects, particularly Hemiptera from suborder Cicadomorpha. Species from the family Aphorophoridae (i.e.: *Philaenus spumarius*) has been detected as the major vectors in other areas of Europe, such as Italy. In this study, we analyse the polymorphism pattern of the potential vector species of *X. fastidiosa* found in Majorca, as well as the bioecology of the nymphs found in the vegetation associated to the crop. For this purpose, different crop plots were selected for sampling insects in Majorca, including almond, citrus, olive and vineyard. Sampling of immatures was carried out from March to June 2017 by direct observation of the cover vegetation. For adults, sweeping net from cover vegetation and vacuum on trees were used and the sampling is still ongoing biweekly. Collected insects were identified in the laboratory using European Keys for families Aphorophoridae and Cercopidae. Insects were also grouped by its colour pattern according to the different forms described in the literature. The majority of insects that were captured were from the family Aphorophoridae and included the genus *Philaenus* and *Neophilaenus*. We present preliminary data on the species composition of potential vector species of *X. fastidiosa* in Majorca as well

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## 5.6 Presence and seasonal appearance of Aphrophoridae, Cercopidae and Cicadellidae in Greece

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**Abstract:** Xylem sap feeding insects of the suborder Auchenorrhyncha are exclusively responsible for the transmission of *X. fastidiosa* in the field. The recent outbreak of the disease in Apulia region in southern Italy has boosted the interest for the study of this group of herbivorous insects. Although, the fauna of Auchenorrhyncha in Greece has been studied in the past (Drosopoulos 2003), there is limited information about their association with olive orchards both in olive trees canopy, ground and surrounding vegetation. In 2017, within the XF-ACTORS project we will survey olive orchards for the presence and seasonal phenology of xylem sap feeding species of the suborder Auchenorrhyncha. Faunistic surveys are performed in four selected regions of Greece (Attica, Arta, Corinthos and Aitolokarnania). Sampling is done three times of the year by sweepnetting the ground vegetation and the olive trees, and by observation and collection of immatures staged of spittle bugs. Additionally, following the same methods of sampling, surveys were also performed in citrus, vineyards and pomegranates orchards which were close to olive-growing area. In one location, sampling at regular intervals will provide information about the seasonal occurrence of these species in olive orchards. Our results will provide insights about the presence of potential vectors in olive orchards in Greece and their seasonal phenology.

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## 5.7 Climatic cues related to the distribution and abundance of candidate vectors of *X. fastidiosa*

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**Abstract:** The vector-borne bacterium *X. fastidiosa* poses a serious threat to the Mediterranean agriculture. Therefore, a deep knowledge about presence, biology and ecology of candidate vectors (Hemiptera: Cicadomorpha) is required to assess the risk of pathogen spread. We sampled olive canopies and the associated ground vegetation in 14 and 16 olive groves in 2016 and 2017 respectively, two of them from the infected area in Mallorca. Olive groves grown under different climatic conditions and cropping practices: organic, conservation tillage and conventional tillage. We observed that *Philaenus spumarius*, the main vector species in Italy, was mainly associated to humid areas where natural perennial vegetation is present most time of the year, mostly in the sierra of

Sevilla and Mallorca. *P. spumarius* were almost absent in regions with conventional tillage such as the "Campiña" of Jaén and Córdoba. Furthermore, we collected another xylem feeder, namely *Neophilaenus campestris* mainly on grasses in Madrid, Córdoba and Mallorca. Data gathered on the population dynamics of *X. fastidiosa* vectors will be analyzed in relation to climatic variables, as the first step to develop a risk assessment model applicable to the sampled regions. Moreover, our data on the spatial distribution on aggregated behavior of the spittlebug species will be useful to develop an environmentally sustainable *X. fastidiosa*-disease mediated control strategy.

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### 5.8 DNA barcoding to identify vectors of *X. fastidiosa* in the Balearic Islands

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**Abstract:** *X. fastidiosa* has recently being detected in the Balearic Islands. Although three of the current varieties of this bacteria has been recorded, there is not yet definitive information on the vectors that transmit this disease in the Balearic archipelago. The DNA-barcoding region is located in the cytochrome oxidase 1 gene (CO1). This has shown efficient to differentiate animal species and it has been widely used in the identification of disease vectors. We propose to standardize the methodology and the selection of the fragment to be amplified to homogenize results obtained by different groups.

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### 5.9 Predatory potential of two functional groups of spiders on *Philaenus spumarius*

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**Abstract:** There is an urgent need of increasing the knowledge about natural enemies of *Philaenus spumarius* and finding strategies to limit the spread of *X. fastidiosa*. Generalist predators, such as spiders, can arise as potential natural control agents. Determining the functional response is crucial for understanding the potentiality of a predator as biological control agent. In this work, the functional response of two widespread palearctic spider species belonging to two different functional groups was assessed. The ambusher spider *Synema globosum* and the orb-weaver *Araniella cucurbitina* were used as model species and *P. spumarius* as prey under laboratory conditions. In parallel, *Ceratitis capitata* was also used as prey in order to compare the spiders' predatory potential between a non-flying insect (*P. spumarius*) and a flying one (*C. capitata*). *A. cucurbitina* and *S. globosum* showed a type II and type I functional response respectively when fed with *P. spumarius* and a type II response when fed with *C. capitata*. Both the handling time and attack rate were significantly different between spider species when fed with *P. spumarius* ( $p < 0.01$ ) and between prey types for each spider functional group ( $p < 0.01$ ). The results suggest that *S. globosum* was more efficient capturing spittlebugs one by one than *A. cucurbitina*. On the contrary, *A. cucurbitina* was more efficient killing flies in webs than *S. globosum*.

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### 5.10 Investigations on dispersal capability of *Philaenus spumarius* by mark-release-recapture method

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**Abstract:** *Philaenus spumarius* is the vector of the CoDiRO strain (subsp. *pauca*) of *X. fastidiosa* in the Apulian olive orchards (Saponari et al. 2014). Dispersal capability of the vector is of capital importance to predict future spreading of the pathogen, thus representing a research priority (EFSA 2016). Mark-release-recapture (MRR) is the method of choice to study movement of vector insects, including *Homalodisca vitripennis*, the main vector of *X. fastidiosa* in South California (Northfield et al. 2009, Blackmer et al. 2006). Although few information on the flight distance covered by *P. spumarius* are available (Halkka et al. 1971, Putman 1953, Weaver and King 1954), none have been obtained with a scientifically sound experimental plan. An experiment was conducted in Piedmont Region, with 650 adult insects marked by a solution of albumin, and released in a single point in a meadow. Spittlebugs were recaptured by sweeping net in 8 directions radiating from the release point up to 200 m, for a total of 92 collection points. Recaptures were conducted up to 15 days after the release. Marked insects were identified via an indirect ELISA. Twenty marked individuals were found within a maximum distance of 60 m. Two similar experiments were conducted in an olive orchard in the Apulia Region, with a total of 2500 marked insects released. Insects were then collected up to 30 days after, by sweeping net on the canopy of olive trees around the point of release up to 120 m in 170 collection points. Five marked insects were recaptured, with the most distant specimen at 100 m. Studies are ongoing during 2017.

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### 5.11 Further physical chemical control tools versus juvenile spittlebugs

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**Abstract:** *Philaenus spumarius* Linnaeus, 1758, (Hemiptera Aphrophoridae) AKA the Meadow Spittlebug belongs to a Taxon whose juvenile live protected into a foamy mass they inflate starting from external glandular secretions and anal excretions. *P. spumarius* control was almost out of the question until the demonstration of its ability to transmit the *X. fastidiosa pauca* strain responsible for the Apulian Olive Quick Decline Syndrome (OQDS) epidemics. We consider here a combination of physical action (hot 60-90°C steam) and a biocontrol insecticide (ABP-617, tradename Flipper by AlphaBio Control Ltd.) of vegetal origin based on carboxylic acids (C14 – C20) potassium salts. Steam originated from an MM STEAM 100-12V weed control machine equipped with a hand shielded steam lance and the insecticide was poured into the water tank at 0.1 % v/v (498 mg of a.s./l of water). Trials were conducted on May 8th, 2017 near Somaglia (LO) in a uncultivated area and we used a randomised block scheme with a 0.25-m<sup>2</sup>-wide test and three replicates, each 0.25-m<sup>2</sup>-wide. During the action, the lance distributed steam plus insecticide over the block for 4'43" consuming 4-5 litres of water from the tank, about 1 lt/m<sup>2</sup>, that corresponds to an insecticide rate of 10 l/ha. Infestation on the test was 20-28 spittles per square meter, but we found neither spittle nor nymphs in treated repetitions. Finally, we discuss pros & cons of the proposed integrated control action.

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### 5.12 Improved juvenile spittlebugs populations quantitative sampling

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**Abstract:** Spittlebugs (Hemiptera Aphrophoridae) have been rising celebrity as one of the, *Philaenus spumarius* Linnaeus, 1758, shown to be the vector of *X. fastidiosa pauca* in the Apulian OQDS epidemics. The juvenile vector population size knowledge is one of the crucial data in establishing an effective control strategy while choosing action thresholds, tuning control action intensity and estimating control efficacy. To quantify at our best the juvenile population size, we realised conventional and innovative spittle sampling. A five-person squad sampled weekly from February to May 2017 in three different field management models: uncultivated, no-tillage and conventional tillage olive orchards. The squad sampled 4,5 m<sup>2</sup> exploring one management per day/week by 18 random transect of 0,25 m<sup>2</sup> each. Our sampling was destructive, contrary to the conservative EFSA-funded field surveys, which implicated the field collection of the plants in transect. For conventional sampling, collected plants were scrutinised in the lab counting juveniles and storing them in 75% EtOH. In case of conventional sampling, each SSU required about 80 minutes of worktime. The innovative sampling method consists in a triple sequential rinsing of collected plant in saline solution. The washing dissolves the spittle and pick up the juveniles to be filtered. This innovative technique, we call AquaSamPling (ASP) has proven to be 20-50% more effective than the conventional one.

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### 5.13 Isoclast™ active as a new tool for controlling *X. fastidiosa* invasion via vector control

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**Abstract:** *X. fastidiosa* is a quarantine bacterium species invading Europe and recognised as the agent of 'Olive Quick Decline Syndrome' (OQDS) disease and which has already killed hundreds of thousands of olive trees in southern Italy. The Palearctic spittlebug *Philaenus spumarius* (Hemiptera: Aphrophoridae,) has been identified as the major vector of this bacterium in Italy being frequently encountered and widely spread in Europe. Juveniles are abundant in spittle on herbs either in natural, agricultural production or urban environments. The spittlebug becomes infective as an adult when it moves to the olive trees during the spring – summer and is exposed to the bacterium within infected trees. Current control measures focus on the mechanical control of juveniles on weeds in early spring and later on the insecticidal application on the olive trees against the mass-spreading adults. The effective and fast control of adult vectors is crucial to prevent or at least mitigate the acquisition of the bacteria and to avoid the subsequent spreading of the infection and eventual damage. Closer (Isoclast in 240 SC formulation) tested in several labs and open field trials effectively controlled *Philaenus spumarius*. This effect is achieved by a very fast feeding cessation followed by the pest mortality resulting in low bacterial infection, higher yield and a healthier plantation in comparison to the untreated.

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### 5.14 Morphometric comparative study of the foregut of two *X. fastidiosa* vectors

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**Abstract:** *X. fastidiosa* attaches to and persistently colonizes the cuticular lining of the foregut of insect vectors (Purcell et al. 1979). Although much has recently been learned about *X. fastidiosa* colonization of vectors, many questions still exist. Among those are the factors determining *X. fastidiosa* populations size in vectors. Estimates based on quantitative PCR indicate that *X. fastidiosa* populations in one vector, *Graphocephala atropunctata* (Cicadellidae), reach ~50,000 cells, while in another, *Philaenus spumarius* (Aphrophoridae), estimates are 1-2 orders of magnitude smaller (Cornara et al. 2016). We hypothesized that foregut (precibarium and cibarium) dimensions in *G. atropunctata* are larger than *P. spumarius*, allowing for larger *X. fastidiosa* populations to establish. A series of morphometric comparisons between the species were performed using micro computer tomography scanning (micro CT scan) as well as scanning electron microscopy (SEM) to test this hypothesis. Results indicate that *P. spumarius* could harbor twice as many cells as *G. atropunctata*. These data suggest that fluid dynamics and not cuticular surface available for *X. fastidiosa* colonization are responsible for the bacterial population size discrepancy observed.

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### 5.15 Seasonal distribution of *Philaenus spumarius* and *Neophilaenus campestris* in Apulian olives groves.

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**Abstract:** A two years investigation was performed in Apulia region to study the population in olive groves of *Philaenus spumarius* and *Neophilaenus campestris*, the vector and the putative vector of *Xylella fastidiosa*, respectively. Adults of these two species were monthly collected from ground vegetation and olive canopy in four severely infected olive groves. This study showed that adults of both species were present from May to December. During this period abundance of *P. spumarius* was constantly increasing to reach its peak in September with no significant difference between ground and canopy levels. Conversely, the pick of population of *N. campestris* was noticed in May-June with a significant decrease until December due to the absence of grassland. However, the latter was greatly preferred by *N. campestris* with respect to the olive canopy. Our findings confirm that weed control in olive groves during Spring could reduce the population of both species. Interestingly, this study shows that treatments against adults of *P. spumarius* should be applied at canopy level during Summer months.

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### 5.16 Effect of different plant species and temperatures on the survival of *Philaenus spumarius*

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**Abstract:** The spittlebug *Philaenus spumarius* (L.) has been identified as vector for the damaging bacterium *X. fastidiosa* in Europe. The knowledge about this insect biology and survival developing on native plants from Europe is crucial to establish ground cover management strategies in olive orchards to control the *X. fastidiosa* spread. In this study we aimed to evaluate the survival of adults developing on two native and common plants in Europe as well as the effect of different temperatures on the *P. spumarius* survival. The species selected for the survival analysis were *Medicago arabica* (L.) Huds. and *Sanguisorba bavarica* (Link ex G. Don) Ces. These plants are distributed all across Europe and presented foams near the campus, where they were collected. New born adults (<24h) were used for survival analysis. Six treatments (*M. sativa* 21°C, *M. sativa* 25°C, *S. verrucosa* 21°C, water 21°C, water 25°C and water 4°C) with 21 to 30 replicates were accomplished. Results indicated that survival of *P. spumarius* could improve when developing on different plants species. Variation in temperatures also affected the *P. spumarius* survival, being particularly remarkable the improvement of survival under 4°C when compared with the other water treatments.

#### Acknowledgements

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### 5.17 Molecular detection of *Philaenus spumarius* DNA in predators gut

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CIMO, School of Agriculture, Polytechnic Institute of Bragança, Bragança, Portugal.

**Abstract:** *X. fastidiosa* is one of the most destructive pathogens of olive orchards. Its recent arrival to Europe can cause important losses, with possible drastic consequences for the sector. *Philaenus spumarius* (Linnaeus 1758) was identified as vector of *X. fastidiosa* in Europe and finding candidate natural enemies of *P. spumarius* is essential to design environmentally friendly control strategies against *X. fastidiosa*. Generalist predators, such as spiders, arise as important potential biocontrol agents. In this context, a set of species-specific primers for detecting the presence of *P. spumarius* DNA in the gut of predatory arthropods is being developed.

All primers will be tested for checking cross-reactive amplification of arthropods DNA and evaluated in heterospecific mixes of nucleic acids. Subsequent feeding trials will be conducted using *Synemaglobosum* (Fabricius 1775), an important spider species in olive groves from Mirandela, Portugal. These trials will allow determining the detection efficiency of the primers designed and also they will be the starting point to detect predation in field trials. These primers therefore provide a very useful tool for screening the gut contents of potential predators of *P. spumarius*, and can thus reveal candidate species for this species biological control.

#### Acknowledgement

This work has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N. 727987 "Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy XF-ACTORS".

## Session 6 – Detection and identification

### 6.1 Internal controls and digital PCR supporting reliable detection of *X. fastidiosa*

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**Abstract:** Official detection of *X. fastidiosa* is done since 2005 using qPCR as screening tests (Schaad *et al.* 2002, Francis *et al.* 2006, PM 2016, Dreo *et al.* 2006). Since then the range of matrices has significantly extended. To assure the reliability of testing several critical issues were identified including (i) utilization and preparation of spiked controls with defined concentrations of the target and (ii) inhibition of amplification in some matrices. In total more than 130 spiked controls were analyzed in 2016 representing *Olea* (31), *Nerium* (24), *Rosmarinus* (24) and *Coffea* (13), and 46 samples of 21 other plant genera and insects. DNA was extracted using automated Quick Pick Plant Kit (Pirc *et al.* 2009) and tested undiluted, gave negative result in 19 (14 %) and 24/138 (17 %) of spiked controls in Shaad's and Francis' qPCR respectively. Samples with most inhibition were of *Rosmarinum*, *Lavandula*, and *Origanum* (14), *Prunus* (2), *Coffea* (2) and individual samples of *Nerium*, *Olea*, *Rubus* and *Hedera* plants. While these controls were positive when tested in dilutions (1:10) the results nevertheless identify matrices for which improvements of sample preparation and/or DNA extraction are necessary. COX amplification (Körner *et al.* 2017) also failed in 10/24 samples making it a relatively good predictor. Digital PCR allowed us to identify (i) its comparable sensitivity to qPCR and (ii) absolute quantification of extracted DNA as affected the matrices. Digital PCR has been reported to improve sensitivity and to have higher resistance to inhibition in plant samples [Dreo *et al.* 2014, Rački *et al.* 2014, Gutiérrez-Aguirre *et al.* 2015].

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## 6.2 Development of an agar absorbent assay and Bio-real- time PCR for the detection of *X. fastidiosa*

Fatmi M.\*, Damsteegt V. D., Schaad N. W.

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**Abstract:** Application of polymerase chain reaction (PCR) to disease diagnosis is limited in part by PCR inhibitors. Inhibition can be overcome, and sensitivity increased, by culturing bacteria on agar media prior to PCR. An agar absorption and Bio-real-time PCR method to detect *X. fastidiosa* in grape and citrus plants was developed. Optimum lengths of time for inhibitors absorption by agar medium or enrichment on the medium were determined for Pierce's disease and citrus variegated chlorosis. When petioles of grape and citrus leaves with symptoms were spotted onto PD2 agar media, the spots washed after various time intervals and assayed for *X. fastidiosa* by real-time PCR, 97% and 100% of spots were positive after 2 days and 4 h for grape and citrus, respectively. With direct real-time PCR only 12.5% and 33% of spots were positive, respectively. Visible *X. fastidiosa* colonies were evident after 10 and 14 incubation days, respectively. In a separate experiment with samples from a different vineyard, 46% of samples (agar spots) were positive after 1 day, and 93% after 5 days using agar absorption real-time PCR. In contrast, all samples were negative by direct real-time PCR. Viable *X. fastidiosa* cells were recovered from all samples after 14 days. Further tests with 8 randomly selected grape petioles from three Texas vineyards known to have Pierce's disease resulted in 50% being positive by a simple 1 day agar absorption real-time PCR assay but none were positive by direct real-time PCR.

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## 6.3 Development of diagnostic assays for the identification of *X. fastidiosa* sub-species

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**Abstract:** The taxonomy of *X. fastidiosa* is complex; sub-species level descriptions are used and are of relevance due to variations in plant host specificity, vectors and geographic distribution between the sub-species. For this reason, the sub-species of any given interception or outbreak is of interest

from a plant health policy perspective. Reliable identification to the sub-species level requires time-consuming DNA sequencing of at least 7 genes (using MLST), a process which takes several days. The development of rapid sub-species identification assays would have a significant impact on the services provided by a diagnostics laboratory. Comparative genomics can be used to allow the fine differentiation of very closely related species and/or sub-species and can be coupled with targeted assay design strategies to allow the development of highly specific diagnostic assays. By utilising the latest genome comparison techniques and developing software to allow the identification of sections of DNA sequence of defined levels of sequence similarity, a suite of *X. fastidiosa* sub-species specific assays have been designed. Testing has shown that sub-species specific assays have been successfully developed. The developed suite of assays allow the rapid determination of the *X. fastidiosa* sub-species present in unknown samples, resulting in a substantial reduction in the turnaround time for identification to the sub-species level compared to all existing diagnostic methods.

#### 6.4 Rapid DNA test of *X. fastidiosa* from laboratory to field

Li R.\*, Russell P., Zhang S., Davenport B., Eads A., Schuetz K., Berkani S., Amato M.

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**Abstract:** *X. fastidiosa*, a Gram-negative and xylem-limited bacterium, is an important quarantine pathogen in many countries. It infects 368 plant species in 91 families and 279 genera. In recent years *X. fastidiosa* has migrated from its American origin to European countries and caused significant damage on olive trees in southeastern Italy and grave concern to its rapid spread to surrounding areas and countries. In order to meet the demand for testing on site, Agdia has developed a *X. fastidiosa*-specific DNA test using the recombinase-polymerase amplification technology, called AmplifyRP. The assay performs both as a real-time and an endpoint test from a single reaction tube at 39°C for 20 minutes. Reaction template is simply prepared by soaking 50 mg of petiole cross-sections in 0.5 mL AMP1 extraction buffer for 10 minutes or by suspending one culture colony in 100 µL AMP1 buffer. The assay reacts to 28 isolates from grapevine, citrus, olive, almond, coffee, oleander, mulberry, American elm, sycamore, oak, blueberry, and blackberry while consistently detecting 22 and even less copies of spiked *X. fastidiosa* genome in soaking extract (1:10, W/V). No reaction background was observed in host tissues. No cross-reaction was observed to *Xanthomonas*, *Erwinia*, *Pseudomonas*, and *E. coli*. The short reaction time, simple sample prep, and use of a portable heat block or battery operated fluorescence reader makes the *X. fastidiosa* DNA test going from laboratory to field.

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## 6.5 Rapid screening tests for the assignment of *X. fastidiosa* genotypes to a subspecies cluster

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**Abstract:** Until now, different molecular tests can be used to assign novel *X. fastidiosa* isolates to subspecies clusters, among which MLST/MLSA represents the most common method. *X. fastidiosa* outbreaks in EU motivated the search for accurate and faster approaches to differentiate the *X. fastidiosa* isolates. Because MLST/MLSA requires PCR reactions and sequencing analyses, 2 independent approaches were recently developed and implemented for rapid taxonomic assignment of uncharacterized isolates: (1) single-nucleotide primer extension (SNUPE) method that allows to differentiate all subspecies and three genotypes within *X. fastidiosa* subsp. *pauca* including the type-isolate infecting olive in Italy and (2) high-resolution melting (HRM) analysis of the amplicon recovered from the gene encoding the conserved HL protein. Both assays were validated on a larger panel of isolates and proved to clearly differentiate *X. fastidiosa* isolates currently known to occur in the Italian, France and Spain outbreaks. These rapid approaches could represent a useful tool for pre-screening of infected samples to be further analyzed by MLST or whole genome sequencing. In addition alternative genomic regions of *X. fastidiosa* are going to be analyzed to implement approaches aimed to assign genotypes to a subspecies cluster, with the purpose to support a rapid identification of genotypes/subspecies at interception places or when new findings occur in a pest free area

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## 6.6 Ground penetrating radar as a tool for an early diagnosis of olive quick decline syndrome

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**Abstract:** *X. fastidiosa* subsp. *pauca* is responsible for a devastating disease of olive trees in Apulia (south-eastern Italy), characterized by a quick decline syndrome, wilting, and death of the plants. In order to develop a possible indicator that can provide information for an early diagnosis of the disease, a multi-source (manned and unmanned aerial platforms, on-field spectroradiometry, on-field radar platforms, visual agronomic assessment) data integration method is being tested. As first experimental test, trunks and main branches of healthy and diseased olive trees were examined by using the Ground Penetrating Radar (GPR). The conceptual hypothesis underlying the test with the GPR is that this method, based on relative dielectric permittivity ( $\epsilon_r$ ), is very sensitive to water level in the medium. Since *X. fastidiosa* clog the xylem vessels, it is reasonable to suppose that sap movement in the affected trees may be anti-related to the severity of the disease. Therefore, an  $\epsilon_r$  value as lower as greater the disease severity should be expected. In addition, a sap deficiency would cause a simultaneous increase in electrical resistivity. Such circumstances would cause both a higher velocity and lower attenuation of the radar waves. Measurements on olive tree's trunks were conducted, both longitudinally and transversally, using different devices (PulsEko1000, with a 1200 Mhz antenna, and Noggin 500 MHz). Preliminary results seem promising and consistent with the starting hypothesis.

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## 6.7 Improvements for better detection, isolation and characterization of *X. fastidiosa*

Poliakoff, F.\*, Legendre, B., Olivier, V.; Dousset, C., Paillard, S., Molusson, D., Sainte-Luce, A., Juteau, V., Cuntty, A., Germain, J.F., Reynaud, P

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**Abstract:** Since the first outbreak of *X. fastidiosa* on *Polygala myrtifolia* in natural settings in 2015 in France, *X. fastidiosa* has been detected on more than thirty plant species with a validated method based on Real-Time PCR (Harper *et al.*, 2010) associated to DNA extraction with QuickPick™ Plant DNA kit (Bio-Nobile) and KingFisher™ automate (Thermo Fisher Scientific). The sample preparation and isolation performed on modified PWG medium (EPPO, 2016) have been optimized and more than 40 *X. fastidiosa* strains were isolated from coffee plants, various ornamentals and trees. Critical reagents used for characterization of isolates directly on plant and on isolated strains according to a multilocus sequence typing (MLST) (<http://pubmlst.org/X.fastidiosa/>) following the amplification protocol of Yuan *et al.* (2010) were evaluated to optimize EPPO protocol PM 7/24. Thus isolates in France were mostly allocated to sequence types ST6 and ST7 (subspecies *multiplex*) although other subspecies were identified punctually (Denancé *et al.*, 2017). *Philaenus spumarius*, known as vector in Apulia is widespread in mainland France and Corsica. A detection method of *X. fastidiosa* in insects was validated based on Real-Time PCR (Harper *et al.*, 2010) performed in duplex with internal controls 18S (Ioos *et al.*, 2009), after DNA extraction using the same commercial kit (Bio-Nobile). The rate of contaminated insects based on individual insect testing varied, according to the outbreak locations in Corsica, from 4% to 25%.

### Acknowledgment

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## 6.8 The innovative plant sap extraction method for pathogen diagnosis: the case of *X. fastidiosa*

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**Abstract:** The extraction of plant pathogens from plant tissue for diagnosis purpose remains one of the key factors for a successful and sensitive detection. Most of the extraction protocols for plant



pathogens suggests grinding plant tissue, eventually in liquid nitrogen. In this case, the final extract is full of several plant components (e.g. polyphenols, plant metabolites) which can inhibit the enzyme activity or affect the diagnostic results. The patented plant sap extraction method (CIHEAM/MAIB Patent number WO2017017555A1) is aimed to obtain microorganisms or part of them from the plant tissue sample with a low content of plant components. The extraction method is based on a low pressure application of the extraction buffer through the plant vessels at one terminal end of the plant tissue (e.g. a 5 cm twig, the leaf peduncle) using a siring with an adapter for the section size of the sample. The collected sap from the other terminal of the plant tissue is collected for diagnosis purposes. This method compared to conventional extraction techniques (CTAB, commercial kits) gave better results in terms of sensitivity, accuracy (no false negatives), costs, time of execution and skills for the diagnosis of *X. fastidiosa* from olive trees using different detection methods: isolation, ELISA, DTBIA, PCR, Real Time PCR and Real Time LAMP. The use of different plant host species and matrices (branch, leaf peduncle, etc.) is still under evaluation.

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## Session 7 – From field detection to disease dynamics

### 7.1 Model & data based prediction of the future dynamics of *X. fastidiosa* in France

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**Abstract:** The spread of *Xylella fastidiosa*, bacterium detected in Corsica during 2015, might cause a major sanitary crisis in France. It might also have diverse impacts on the French agricultural production through important direct or indirect costs. In this poster, we will present a short-time approach to predict the future dynamics of emerging plant pathogens such as *X. fastidiosa* in France. This would be a major advantage for adopting control and surveillance measures at an early stage. We used several spatio-temporal propagation models to estimate epidemiological parameters and make prediction from surveillance data. These models were built by following a mechanistic-statistical approach, which combines (i) a sub-model based on partial differential equations describing the dynamics of the pathogen and (ii) a stochastic sub-model describing the observation process. To draw a unique prediction of the future extent of the pathogen, we used model-averaging as a way to take account of uncertainty about model forms. We will show the results obtained in the case of the expansion of *X. fastidiosa* in France, but could be applied to other post-emergent epidemics to endorse a fast reaction.

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### 7.2 Assessing the global potential distribution of *X. fastidiosa* using species distribution models

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**Abstract:** Species distribution models (SDMs) determine the relationships between sample location for a species and associated environmental variables, and are used to estimate the ecological requirements for a particular species. SDMs provide realistic scenarios to explain the influence of bioclimatic variables on the epidemiology of plant pathogens, particularly in the context of emerging plant diseases. Different modeling techniques, including regression, classification and machine learning approaches were used within an ensemble forecasting framework (Naimi & Araujo, 2016) to quantify and map the global patterns of the potential geographic distribution of *Xylella fastidiosa*. The global distribution of *X. fastidiosa* was obtained from EFSA (EFSA, 2016). To cope with the equilibrium



assumption, pseudo-absence data were generated outside the organism's ecological domain (Barbet-Massin *et al.*, 2012). Overall, projected potential distribution from estimated models conformed well to the current known distribution of *X. fastidiosa*. The application of SDMs to the most prevalent *X. fastidiosa* subspecies (i.e. *fastidiosa*, *pauca* and *multiplex*) will be discussed.

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## Session 8 – Surveillance and control: from modelling to stakeholders analyses

### 8.1 Risk assessment for possible establishment of *X. fastidiosa* in Bulgaria - host plants and vectors

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**Abstract:** Some of the potential host plants and vectors of *X. fastidiosa* are distributed on the territory of Bulgaria and this creates a risk of spreading in the country. The establishment of the species composition of the potential vectors of the bacterium are based on surveys, conducted in the risky areas of the country.

The possible penetration and spreading of *X. fastidiosa* on the territory of Bulgaria can lead to serious economic consequences as some of the host plants are economically important crops in the country, which occupy an important part of the forest ecosystem and urban vegetation. Potential vectors of *X. fastidiosa* in Bulgaria are 27 species of 6 families.

Since 2002, observations on the presence of *X. fastidiosa* in Bulgaria have been made on vine – in vine nurseries, in plantations with imported and local propagating material, and periodic observations are carried out from 2014 on a wide range of host plants. Between July 2014 and June 2016, 2268 visual inspections were performed on the territory of Bulgaria and 505 samples of *X. fastidiosa* host plants were tested. The largest share of inspections and tests were carried out in vineyards followed by fruit trees. At this time, the bacterium is not registered either on the territory of the country or on imported plant material.

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### 8.2 Management of *X. fastidiosa* surveillance data in France

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**Abstract:** Management of *X. fastidiosa* surveillance data in France, a proof of concept for the national epidemiological surveillance platform for plant health. Data management is a key issue in epidemiological surveillance systems to follow the sanitary situation and to improve the mobilisation of the surveillance network. The surveillance of *X. fastidiosa* in France produces lot of data on the samples, their localisation and on the analysis results. In order to optimise the data collection and management, an IT-system was developed at the national scale. After verifying the data quality, data are stored in a database. The data collected are then used to analyse and evaluate the data quality and the surveillance system. Results are discussed within a multi-stakeholder working group to improve the surveillance procedures. Moreover, two web applications were developed and are automatically and regularly updated. The first one provides precise and reliable information and analyses of the epidemiological situation to the official phytosanitary services and the research. The second one is in open-access and presents the restricted zones. This approach has a pioneer role for the national epidemiological surveillance platform for plant health, which is a public-private partnership under construction. Its aim is to share a common methodology for surveillance of harmful organisms, to improve surveillance and identify research questions.

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### 8.3 Improving monitoring activities on *X. fastidiosa* by sharing laboratory capacities within Europe

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**Abstract:** Recently, several outbreaks of *X. fastidiosa* are threatening agriculture and ornamental plants in Europe. The entry pathways and the means of spreading are not yet fully elucidated. The strongest evidence for the pathogen's movement and dissemination is the trade with infected plants for planting. As the list of host plants is enormous and still increasing due to new findings, monitoring activities became challenging lately. Especially MS that either have significant trade with relevant plants, or are affected by outbreaks or do not possess the by the EPPO recommended testing procedures in place, will eventually outsource their testing to other MS labs. These labs, if equipped to perform the testing according to the state of the art could well cooperatively provide this quality service. Such a course of action will definitely improve the precision and scale of testing, impeding that *X. fastidiosa* is transferred to areas within the EU territory where it was not detected before. The Austrian plant health laboratory is involved in such an action, providing testing capacities to other MS within monitorings for *X. fastidiosa*. Since 2015 more than 700 samples were screened for the presence of *X. fastidiosa* in about 10 important host plant species for 3 MS. Furthermore, it is a great possibility for testing the robustness of detection methods gaining more experience and improving the performance data of the established testing procedures also as a feedback for revisions of common diagnostic protocols.

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#### 8.4 Results of a survey for early detection and prevention of entry of *X. fastidiosa* in Greece

Holeva M\*, Karafla C, Glynos P, Siderea E, Togias A, Goumas D, Trantas E, Ververidis F, Akrivou A, Antonatos S, Evagelou V, Kapantaidaki D, Milonas P, Kagias J, Ioannidou S, Arampatzis Ch, Papachristos D

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**Abstract:** After the first outbreak of *X. fastidiosa* in Europe and the high risk for its entry in new areas, phytosanitary checks have been intensified in Greece on imported or domestic plant material and putative insect vectors. The National Pest Survey Program, co-financed by the EU, involves plant monitoring and sampling based on the EU guidelines, EPPO standards, EFSA Scientific Opinions (1,2,3,4,5,6) and relevant data from scientific projects on *X. fastidiosa* mainly with regard to its host plants and insect vectors. Given the wide host range of *X. fastidiosa* and the broad occurrence of host plants in the country, the survey's priorities are places at higher risk, such as harbours and airports, as well as those host plants which either are of major economic importance for Greece, or constitute a great part of consignments usually being imported, or exhibit susceptibility and a high frequency of infection, such as polygala, myrtle and coffee plants. Laboratory assays (2) on symptomatic/asymptomatic plant samples and putative insect vectors, collected by the Phytosanitary Inspectors, gave negative results as far as plant infection or insect infestation are concerned. In this communication, we present the geographical distribution of areas monitored together with the plant and putative vector species tested, in conjunction with data on the laboratory assays used to confirm plant health status and absence of *X. fastidiosa* in vectors. Future survey strategies to prevent entry and spread of *X. fastidiosa* are discussed.

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#### 8.5 Developing a multicriteria approach to prioritize EU plant pests based on socio-economic and environmental impacts

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**Abstract:** This paper describes the development of a methodology to support the identification of priority pests at EU scale by means of quantification of the severity of their economic, social and environmental impacts. We use a combined methodological approach of multi-criteria decision analysis (MCDA) with composite indicators to provide an estimation of the impacts for a given pest. MCDA is an advanced field of operations research and management science, devoted to the development of decision support tools methodologies. Composite indicators are based on sub-indicators that have no common meaningful unit of measurement, as for example the sub-indicators needed for measuring each of the criteria set forward in Regulation 2016/2031. For each of the points mentioned in Regulation EU 2016/2031 that need to be fulfilled to qualify as priority pests quantitative or qualitative

indicators are identified and measured and the relative importance weights estimated. This approach will be applied to the *X. fastidiosa* case study, among other pests, and will be built on the current and available scientific knowledge. Our findings can help to support plant health preparedness both at EU and Member State level, with more prevention, detection and control.

### 8.6 Monitoring of *X. fastidiosa* in the pathogen-free area using the spy insects approach.

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**Abstract:** Following the strategy of the 'spy insects' for assessing the presence of *X. fastidiosa* in the pathogen-free areas before symptoms development in olive trees, an official monitoring was carried out in the surveillance area, surrounding the buffer zone of Apulia region, in the period June-October of 2015. Adults of *Philaenus spumarius*, the assessed vector of *X. fastidiosa*, and *Neophilaenus campestris*, a putative vector, were collected mainly with the sweeping nets in the provinces of Brindisi and Taranto. Following the cartographic grid of the surveillance area, 41 meshes were selected for the presence of olive groves and main communication roads, representing about 39,000 ha. A total of 925 sites were selected and georeferenced using the application XylApp. About 2,500 "spy insects" were collected in a radius of about 20 meters from each georeferenced point, maintained in coded tubes with ethanol and sent to the laboratory for testing by real time LAMP using the commercial kit (Enbiotech – Italia).

*X. fastidiosa* was detected in 92 specimen, 90 of *P. spumarius* and 2 of *N. campestris*, which were collected from 21 sites. Only one infected site was in the eastern part of the surveillance area, while the remaining were concentrated in the western side. These results were also confirmed by the detection of infected trees during the monitoring of host plants conducted in 2016 in the surveillance area (subsequently demarcated as containment area by the EU decision 764/2016).

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### 8.7 Infoxylella.it: an experience of information and dissemination for a difficult topic

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**Abstract:** Infoxylella.it is a spontaneous initiative born in February 2016 by a group of researchers, technicians, olive growers, etc., all somehow involved in the *Xylella* topic, aimed to contrast conspiracy, disinformation and confusion generated, above all through social media, and partly responsible for the opposition and slowdown in the application of containment mandatory measures defined at European level. Infoxylella wants to communicate to and inform transparently public opinion and media with a simple and immediate language. In addition to the website ([www.infoxylella.it](http://www.infoxylella.it)), the editorial team (more than 30 people), manages a Facebook page and a Twitter profile that in 14 months saw a huge increase in the number of users. The constant commitment and the rigid rules on the dissemination of information only from official and verified sources have progressively increased the credibility and visibility of infoxylella that is often cited or used by journalists and media, like a press agency, as source of information. Although all contents are in Italian, thanks to the online translation utilities, infoxylella has today more than 4800 faithful followers in 45 different countries and a monthly average of 50.000 peoples reached. On behalf of the young experience of infoxylella, we believe that information, together with research results, can play an essential role in the effective application of the containment measures against *Xylella* and the epidemics in Europe

## 8.8 Challenges and opportunities for raising awareness of *X. fastidiosa* within UK horticulture

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**Abstract:** Horticulture contributes £9 billion to the UK economy each year, employing approximately 300,000 people across a range of disciplines (Ornamental Horticulture Roundtable 2015). There are over 20 million gardens in the UK, accounting for 4% of UK land area. Gardens play an important role in supporting biodiversity and provide numerous socio-economic benefits. New and emerging pathogens, in particular *Xylella fastidiosa*, therefore have potential for devastating negative impacts on the economy, the environment, and health and wellbeing (Defra 2014). The Royal Horticultural Society (RHS) is a charitable organisation whose vision is to enrich everyone's life through plants and make the UK a greener and more beautiful place. The charity has approximately 480,000 members and distributes advice and information through a monthly magazine, the RHS website, emails, and a freely available diagnostic service to its UK members. In the last year, over 80,000 enquiries were received by the RHS with around 5% requiring expertise from pathologists. This places the RHS in a unique position to educate the UK horticultural community, and to monitor the emergence and spread of pathogens and detect host shifts.

*X. fastidiosa* is an emerging disease in Europe, but the UK public and horticultural community have little knowledge of this pathogen or the symptoms associated with it. This poster will discuss the challenges and opportunities which the RHS has encountered when raising the awareness of *X. fastidiosa* within UK horticulture.

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## 8.9 Plant pests survey guidelines

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**Abstract:** The European Commission is requesting EFSA to facilitate the Member States in their planning and execution of their survey activities, in particular, EFSA is asked to provide scientific and technical guidelines in the context of the new plant health regime, Regulation (EU) 2016/203, where prevention and risk targeting are given an extra focus and the European Commission co-financing programme of the annual MS survey activities for pests of EU relevance, Regulation (EU) No 652/2014.

In order to address this mandate EFSA will deliver by the end of 2019: (i) 50 pest specific data-sheets that contain practical information required for preparing survey design; (ii) tailor to plant health existing statistical tools at EFSA to estimate the sample size and design the sampling strategy (e.g. RiBESS+ & SAMPELATOR); (iii) survey guidelines for 3 different pests that will be case studies to be developed in collaboration with the EU member states; (iv) training to the member states on underpinning statistics and on the use of the tools, and; (v) the review and adaptation if needed of the existing EU guidelines for surveillance of *Xylella fastidiosa*.