European Food Safety Authority

3rd European conference on Xylella fastidiosa 2021

XF ACTORS

Xylella Fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy

3rd European conference on *Xylella fastidiosa* and XF-ACTORS final meeting

Building knowledge,
protecting plant health

26-30 April 2021

Book of Abstracts

draft

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XF-ACTORS Final Meeting

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Introduction and adaptation of an emerging pathogen to olive trees in Italy

Sicard A[1,2], Saponari M[3], Vanhove M[1], Castillo A I[1], Giampetruzzi A[3], Loconsole G[3], Saldarelli P[3], D[3], Neema C[2], Almeida RPP[1]

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The invasive plant pathogen *Xylella fastidiosa* currently threatens European flora through the loss of economically and culturally important host plants. Previously absent from Europe, and considered a quarantine pathogen, *X. fastidiosa* was first detected in Apulia, Italy in 2013 associated with a devastating disease of olive trees. Although the biology of *X. fastidiosa* has been studied for over a century, there is still no information on the determinants of specificity between bacterial genotypes and host plant species, which is particularly relevant today as *X. fastidiosa* is expanding in the naïve European landscape. We analyzed the genomes of 79 *X. fastidiosa* samples from diseased olive trees across the affected area in Italy as well as genomes of the most genetically closely related strains from Central America. We provided insights into the ecological and evolutionary emergence of this pathogen in Italy. We showed the pathogen was recently introduced, and we generated a list of candidate genes that could play a major role in the adaptation of *X. fastidiosa* to new environments.

Keywords: population genomics, adaptation, Olive Quick Decline Syndrome

Probable dates and scenario of introduction of *Xylella fastidiosa* subsp. *multiplex* in France

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Xylella fastidiosa was detected for the first time in the French territory in 2015, in Corsica and PACA regions. Most samples were infected by strains from two lineages (ST6 and ST7) of *X. fastidiosa* subsp. *multiplex*. While numerous of foci were detected all over Corsica, in natural, urban and semi-urban environments, in a large range of hosts (39 plant species), a more limited number of mostly urban foci was reported from PACA in a range of 25 plant species, from which 15 were not reported infected in Corsica. Hence, situations look like having different histories. Our aim was to decipher the most probable scenario of introduction of each ST in these two regions. A collection of 82 genome sequences including 56 genome sequences of French strains was used to date the divergence of French *X. fastidiosa* subsp. *multiplex* strains from their American relatives. We developed a 13-VNTR scheme for direct typing of *X. fastidiosa* subsp. *multiplex* in infected plant samples and used Approximated Bayesian Computation analyses to select the best scenario to explain the spread of the bacterium in France. The scenario choice and testing will be presented together with parameters allowing the selection of the best scenario and its interpretation in the context of plant trade.

Extreme temperature differentially affects growth and survival of *Xylella fastidiosa* strains

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The development of regionalised risk models for the establishment of *Xylella fastidiosa* (*Xf*) and its subspecies requires a better knowledge of their respond to environmental factors in particular to temperature. Furthermore, since *Xf* is confined within the xylem, it will be relevant to determine to which extent the xylem environment differs from external conditions. Therefore, the objectives of this study were to determine the effect of temperature in growth and survival of *Xf* strains and to establish the dynamics of temperature in xylem vessels in olive trees. Growth and biofilm formation of 33 *Xf* strains representative of five subspecies and 13 STs, a wide geographic origin and hosts plants have been determined in vitro in a temperature range from 4 to 40ºC. After 7 days, planktonic cells were inoculated into 96-well plates and incubated at 28ºC for 7 days more. On the other hand, air and xylem vessels temperature was measured at 10-min intervals during 31 months by installing thermocouples in soil, branches and trunks of four 8-yr old olive trees at a 1 and 4 cm depth.

Our results indicated that overall, extreme low or high temperature differentially affected growth and survival of *Xf* strains. Thus, when *Xf* bacterial cells were incubated at 4 to 10ºC showed a slower growth rate and reduced biofilm formation compared to those incubated at 16 to 32ºC. However, cells that grew at all those temperatures were able to growth further and form biofilm when incubated at favourable temperature of 28ºC in fresh medium. In contrast, incubation temperature of 36 and 40ºC for a 7-day period resulted lethal for the bacterial cells since no further cell growth was observed after incubation at 28ºC.

Regression models were developed to estimate the relationship between air and xylem or soil temperature. The slope of the fitted linear models indicated a poikilothermy where air and branch temperature progress at the same rate (slope=1), but a homeothermy in trunks and the soil-root system where air temperature increases at a lower rate (slope < 1). The buffer effect of the trunk and soil are stronger for daily maximum temperature in summer and winter seasons, while minimum daily temperature was coupled with air temperature across seasons. Our results indicate the need to take into account the differences on temperature in the xylem environment when developing regionalized risk models for *Xf* and highlight the importance of the differential effect of low and high temperature on *Xf* growth.

Acknowledgements
This work has received funding from 727987 XF-ACTORS (H2020-UE), E-RTA2017-00004-C06-02 from AEI-INIA Spain and FEDER and the Spanish Olive Oil Interprofessional.

Investigation of the metabolic network of *Xylella fastidiosa* responsible of its fastidious growth

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High proliferation rate and robustness are vital characteristics of bacterial pathogens that successfully colonize their hosts. The observation of drastically slow growth in some pathogens is thus paradoxical and remains unexplained. In this study, we sought to understand the slow (fastidious) growth of the plant pathogen *Xylella fastidiosa*. Using genome-scale metabolic network reconstruction, modeling, and experimental validation, we explored its metabolic capabilities. Despite genome reduction and slow growth, the pathogen's metabolic network is complete but strikingly minimalist and lacking robustness. Most alternative reactions were missing, especially those favoring fast growth, and were replaced by less efficient paths. We also found that the production of some virulence factors imposes a heavy burden on growth. Interestingly, some specific determinants of fastidious growth were also found in other slow-growing pathogens, enriching the view that these metabolic peculiarities are a pathogenicity strategy to remain at a low population level.

Unravelling distinctive features of *Xylella fastidiosa* strain ‘De Donno’

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Several studies have been conducted on the epidemiology of the virulent pathogen *X. fastidiosa* subsp. *pauca* strain ‘De Donno’ that is causing a severe disease in olive trees, but limited data are available on its phenotypic and biological traits. To fill this knowledge gap, the in vitro behaviour of the strain was investigated, exploring relevant biological features, like growth rate, biofilm formation, cell-cell aggregation, and twitching motility, making comparison with the recognised type strain Temecula1, belonging to subsp. *fastidiosa*. The strain ‘De Donno’ had enhanced biofilm production and showed a more aggregative phenotype than the strain ‘Temecula 1’. Specific strains of *X. fastidiosa* have been shown to have natural competence, a biological trait that is related to its ability to uptake the DNA from the external environment. Using the established protocols and in spite of several attempts with different DNA sources the strain ‘De Donno’ failed to show natural competence. This lack of natural competence is consistent with the Citrus-infecting pauca strains. Genetic manipulation was possible with electroporation and mutants were successfully obtained in the presence of an inhibitor of the Type I restriction-modification (T1RM) system. Two different plasmids containing the chromosomal replication origin (oriC) of *X. fastidiosa* and *E. coli*, respectively, were used to transform *X. fastidiosa* ‘De Donno’ strain to produce a GFP-expressing and a knockout strain for *rpfF* gene, an enoyl-CoA hydratase, belonging to the crotonase family enzyme, that is involved in producing a diffusible signal factor (DSF) used for *Quorum sensing*. The outcomes of this study shed light on peculiar traits of the strain ‘De Donno and produced functional mutants that will be useful to gain novel insights into the biology of this strain and its pathogenicity mechanisms.

Keywords: biofilm formation; natural competence; type I restriction–modification systems; green fluorescent protein

Studies on Diffusible Signal Factors molecules in *Xylella fastidiosa* strain De Donno

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Xylella fastidiosa (*Xf*) uses a cell-cell communication system to promote collective behaviors within the population. Such system, named Quorum sensing as it is based on cell density, relies on the production, perception, and response to chemical signals that regulate the expression of genes encoding virulence factors or responsible of the bacterial adaptation to changing environmental conditions. Signal molecules in the Quorum sensing system of Xf, are cis-2-unsaturated fatty acids. Commonly known as Diffusible Signaling Factors (DSF), they share a fatty acid carbon chain with variations in length, double-bond configurations, and side-chains.

To gain insights on the biological features of the Xf strain responsible for the Olive Quick Decline Syndrome, we set up extraction, mechanical treatments, and methyl ester derivatization of the extracted crude oils from cultures. We then compared the GC-MS profiles of extracted and concentrated fatty acids, and taking advantage of 1H-NMR and 13C-NMR spectra. We speculated on obtaining branched-C12 unsaturated fatty acid with a methyl group, and an α-unsaturated moiety. Based on the acquired and already available data on chemical features of DSF molecules in other strains, the synthesis of chemical analogs was carried out with the aim that altering the levels of DSF in plants, may affect population dynamics and reduce the impact of Xf-infections in olives. Four cis-2-unsaturated fatty acids, 2- (Z) -tetradecenoic acid; 2- (Z) -9- (Z) -dodecenoic acid; 2- (Z) -6- (Z) -dodecenoic acid and 2Z, 4E-5-(3-hydroxy 4- methoxyphenyl) penta 2,4 dienoic acid, were ex-novo-synthesized by exploiting the stereoselective Still-Gennari olefination.

The in-vitro analysis of a range of concentrations of these molecules, from 0.1 μM to 100 μM, revealed a complex scenario concerning the role of cis-2-fatty acids in influencing the biological traits of the Xf strain De Donno, like adhesion and biofilm formation.

Artificial inoculation of *Xylella fastidiosa* subsp. pauca strains in olive plants; an overview of greenhouse experiments

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The plant-pathogenic bacterium *Xylella fastidiosa* (*X.f.*) is known to colonize hundreds of plant species but to cause disease in a minor number. Despite its broad host spectrum, some subspecies of these bacteria are restricted to specific hosts. For example, strong evidence of no cross-infection of *X.f.* subsp. *pauca* from citrus was observed in coffee and vice versa by artificial experiments or naturally (Francisco et al., 2017 – Phytophatology). However, the emerging of *X.f.* subsp. *pauca* as a serious pathogen to olive crop and the epidemic characteristic of this subspecies in Brazil whereas a variable number of sequence type (ST) is present highlight the importance to know the response of olive plants to different ST of *X.f.* subsp. *pauca*. On the other hand, for our experience artificial infection of *X.f.* subsp. *pauca* in olive plants has been no efficient reaching the maximum of 33% of success. Based on this background the hypotheses of this work are - i. olive plants can be infected by different STs of *X.f.* subsp. *pauca*; ii. the cultured medium used for bacteria grown (PD3 – complex and XFM – minimum) can affect the inoculation efficiency. By the results of experiments in the greenhouse, we observed that ST11 and ST13 from citrus, ST16 from coffee, and ST70 from hibiscus were able to infect olive plants. The average of positive infection in olive plants by *X. fastidiosa* with 24 h of incubation in XFM, after grown 5 days in PW medium, reached 45% and only 23% with incubation in PD3. By using citrus seedlings as test plants, cells with 24 h of incubation in XFM reached 69% of infection against 52% of grown in PD3. On the other hand, no difference in infection was observed for the medium (87% for both XFM and PD3) when tobacco was used as the plant test. Despite the broad infection by the different STs and the increase of inoculation efficiency, no symptoms were observed in *X. fastidiosa* infected olive plants, so far. By the field observation, symptoms associated with *X. fastidiosa* in olive crops are observed only in plants older than four years old.

Study supported by Projects 727987 XF-ACTORS (EU-H2020) and CNPq 126414-/2019-0.

Xylella fastidiosa subsp. pauca and multiplex elicit differential responses in a susceptible olive cultivar

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The emergence of *Xylella fastidiosa* in Europe revealed the occurrence of bacterial strains belonging to different subspecies and unraveled the vulnerability of some typical European plant species. Among these, olive was found infected in different European outbreaks, with infections caused by strains harboring different sequence types (STs) and belonging to subspecies *pauca* and *multiplex*.

In this work, we compared under greenhouse conditions, the response of the olive cultivar "Cellina di Nardò" to infections caused by the strains De Donno (subsp. pauca, ST53) and ESVL (subsp. multiplex, ST6), as this cultivar is one of the most susceptible in the Italian outbreak. The data showed that: (i) strain De Donno systemically colonized the plants faster than the strain ESLV; (ii) 1.5 years post-inoculation, the bacterial population detected in the plants systemically infected was ~25 times higher for strain De Donno than ESLV; (iii) a severe shoot dieback were detected on the De Donno-infected plants, whereas up to 3 years post-inoculation no dieback and desiccations have been recorded on the ESLV-infected plants. This pathogenicity test was complemented with an RNA-seq analysis. Nine libraries, three for each condition (ESVL, De Donno, mock inoculated), were sequenced by Ilumina technology and sequences mapped against the *Olea europeae* cultivar Picual genome for the analysis of differential gene expression (DEG). The output of this study showed that, respect to the 6,200 genes differentially expressed (FDR<1.00E-05) in De Donno-infected plants, only 550 (FDR<1.00E-05) genes had an altered expression in the ESVL-infected plants.

The present data showed that strains of the two subspecies elicit differential host responses, which were biologically and molecularly attenuated in the ESVL infections with respect to the aggressive De Donno infections and provide useful insights for exploring the molecular basis of *Xylella*-olive interaction.

Keywords: *Xylella*, ESVL, olive

Estimating prevalence and population size of *Xylella fastidiosa* in olive cultivars with differential phenotypic responses to the bacterial infection

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Prevalence and bacterial population size of *Xylella fastidiosa* (*Xf*) were studied in multivarietal olive groves located in the demarcated infected area of Apulia (southern Italy), under high natural pressure of inoculum.

Surveys to assess the occurrence of typical *Xylella*-induced symptoms and the prevalence of infections included the cultivars Ogliarola salentina, Coratina, Frantoio, FS17 and Leccino. Results showed that infection rates varied according to the cultivar, being 40-60% for FS17 and Leccino, respectively, and close to 90% for the remaining cultivars. On a subset of 12 infected olives/cultivar, inspections and sampling were performed in 4 different period of the year, with leaf and xylem tissues tested separately. Symptoms of dessication at the beginning and end of the experiment, scored on a scale of 0 (not present) to 5 (severe), ranged from 4.5 to 5 for Ogliarola salentina, from 3 to 4.5 for Coratina, from 1,5 to 3 for Frantoio, and from 0.5 to 1 for FS17 and Leccino. Diagnostic tests showed that: (i) the bacterial populations of Leccino and FS17 were consistently lower than those of Ogliarola salentina and Coratina (on average 105 vs 107 CFU/g), regardless the period and the tissue; (ii) the bacterium was readily detected (up to 100% of diagnostic accuracy) in the most symptomatic cultivars (Ogliarola salentina and Coratina), regardless the period of sampling and the tissues used; (iii) for Frantoio, FS17 and Leccino, the highest values of diagnostic accuracy were obtained using xylem tissues, without significant differences among the sampling periods; (iv) in FS17 the use of leaf tissues yielded the lowest level of detection accuracy in most of the sampling periods; (v) the trees of Frantoio showed an intermediate behaviour, both for symptoms and bacterial population size.

These results show that quantitative measures of *Xylella* populations correlates with the phenotypic responses to *Xf*, and may help to predict, before the onset of symptoms, the degree of susceptibility of a given olive genotype. Important information for the detection of the bacterium in olives were also obtained.

Keywords: Bacterial population; susceptibility; *Xylella*

Xylella fastidiosa and olive interactions: the key role of the plant cell wall

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Evidences from the Pierce's Disease suggest a major role of the plant cell wall in the mechanism of pathogenesis. In grapevine, *Xylella* knock-out mutants of Cell Wall Degrading Enzymes - CWDE (polygalacturonase, endoglucanase) are impaired in movement and pathogenicity as they are unable to degrade cell wall middle lamellas of Pit Membranes (PMs), porous selecting structures interconnecting adjacent xylem vessels. In olive, repeated studies comparing the transcriptome profiles of cultivars susceptible (Cellina di Nardò) and resistant (Leccino and FS17) to the *Xylella fastidiosa* (*Xf*) strains "De Donno" (XfDD, susbp *pauca*) and CO33 (XfCO33, subsp *sandyi*) unraveled the involvement of cell wall and plasma membrane-located Receptor-like kinases in the plant response to the infection. Members of the Leucin Rich Repeat Receptor-like kinases (LRR-RLKs) and Wall associated kinases (WAKs) are strongly associated with infections of strains XfDD and XfCO33. Both categories of receptors specifically highlight the cell wall and plasma membrane involvement in the pathogenesis of these two *Xylella* strains in olive, as homologous of LRR-RLKs and WAKs are respectively sensors of the cell wall integrity and involved in the plant immune response and resistance to diverse pathogens in *Arabidopsis thaliana* (At). In addition, At WAK1 respond to oligalacturonide fragments released during the pathogen attack by the CWDE activity, which is consistent with the mechanism of pathogenesis of *Xf*. Further evidences that XfDD exploits the CWDE degradation activity to systemically invade olives came from electron microscope observations of the PMs status in infected plants. Micrographs clearly indicate that the pectic and cellulose structures of the primary walls and of the middle lamellas of PMs interconnecting two adjacent xylem vessels, are highly degraded in XfDD-infected Cellina while these are intact in healthy olives.

Keywords: cell wall, receptors, resistance

XF-ACTORS Final Meeting

DAY 2 – Tuesday 27 APRIL 2021 - 14.30-17.40

SESSION 2: *Vectors and control strategies* Chairs: Alberto Fereres (CSIC-ICA) and Micheal Maixner (JKI)	
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14.55-15.10	Diversity of vectors and their role in the spread of *Xylella fastidiosa* in olive orchards of Southeastern Brazil ***Joao R. S. Lopes****, Universidade de São Paulo, Piracicaba, São Paulo (BR)*
15.10-15.20	Q&A
15.20-15.35	New insights on *Xylella fastidiosa* subsp. *pauca* vector transmission to olive plants ***Domenico Bosco****, Institute for Sustainable Plant Protection, CNR (IT) and Department of Agriculture, Forestry and Food, University of Torino (IT)*
15.35-15.50	On the importance of multidisciplinary studies on insect vectors to better understand vector-borne plant diseases ***Astrid Cruaud*** *CBGP, INRAE, Montferrier-sur-Lez (FR)*
15.50-16.00	Q&A
16.00-16.15	Impact of low susceptible and resistant host plants on the transmission of *Xylella fastidiosa* subsp. *pauca* ST53 by *Philaenus spumarius* (Hemiptera: Aphrophoridae) ***Vincenzo Cavalieri****, Institute for Sustainable Plant Protection CNR, Bari (IT)*
16.15-16.30	Vibrational-disruption of the feeding behavior of a plant pathogen vector ***Sabina Avosani****, Department of Civil, Environmental and Mechanical Engineering, University of Trento, Italy and Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige (IT)*
16.30-16.40	Q&A
16.40-16.55	Host plant selection by *Philaenus spumarius*: using ground covers as trap crops ***Marina Morente****, Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), Alcalá de Henares, Madrid (ES)*
16.55-17.10	Defining a set of integrated tools recommended for IPM strategy to control spittlebugs ***Crescenza Dongiovanni****, Centro di Ricerca, Formazione e Sperimentazione in Agricoltura "Basile Caramia", Locorotondo, Bari (IT)*
17.10-17.20	Q&A
17.20-17.40	**Coffee Break**

Feeding habits of Central European candidate vectors of *Xylella fastidiosa* on grapevine

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Xylella fastidiosa subsp. *fastidiosa* is a bacterium native to Central America and causal agent of a disease known as Pierce's disease (PD), whose natural spread only relies on xylem-feeding insects. Within Europe, it has been detected for the first time in Mallorca Island, Spain, in 2017. This first confirmation of PD within Europe raised great concern about the risk posed by the bacterium to the European wine industry. A better understanding of the occurrence and ethology of candidate vectors in European winegrowing systems is a prerequisite to develop effective control strategies against a vector-associated spread of *X. fastidiosa* after its introduction into a new environment.

Therefore, we aimed at (i) characterizing the insect vectors community in Central European vineyards and (ii) analyzing the probing and feeding behavior of prevalent species in order to decipher differences in the transmission potential of *X. fastidiosa* by these candidate vectors to grapevine. Spittlebugs and sharpshooters represented the most abundant vector candidates in vineyards in Germany. Two spittlebugs whose vector competence has been demonstrated in previous studies, the meadow spittlebug *Philaenus spumarius*, currently assumed to be responsible for all outbreaks detected in Europe so far, and *Neophilaenus campestris*, were the most abundant xylem-feeders within monitored vineyards. In addition, two sharpshooters were occasionally collected on vine shoots, the autochthonous *Cicadella viridis* and the introduced Nearctic *Graphocephala fennahi*. The latter, member of the same genus as the American PD-vector *Graphocephala atropunctata*, is a common sharpshooter species in Central Europe and present on grapevine plants located in public parks and private gardens.

We additionally characterized and compared the feeding characteristics of spittlebug and sharpshooter species on grapevine using the EPG (Electrical Penetration Graph) technique. Here we present the main differences in host-insect interaction and their possible reflection on bacterium epidemiology and vector-bacterium relationship.

Keywords: sharpshooters, spittlebugs, vectors

Diversity of vectors and their role in the spread of *Xylella fastidiosa* in olive orchards of Southeastern Brazil

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Olive quick syndrome decline is an emerging disease in Brazilian olive orchards, whose putative etiological agent is *Xylella fastidiosa* subsp. pauca. Since the first report in 2016, the disease has been reported in several locations in the states of São Paulo and Minas Gerais. Surveys with yellow sticky traps have shown a high diversity of potential vectors, including numerous species of xylem-sap feeding leafhoppers (sharpshooters) (Hemiptera: Cicadellidae) and spittlebugs (Hemiptera: Cercopoidea). Among them, 11 sharpshooters and three spittlebugs were shown to transmit *X. fastidiosa* subsp. pauca from infected to healthy olive trees in a greenhouse experiment. Surveys with sweep net showed that some of these species are frequent on the ground vegetation of the orchards, while others inhabit woody species in surrounding areas. Various species were trapped by sweep on the canopy of olive trees, though in very low populations. The occurrence of these vectors either on olives or on surrounding plants, combined with the detection of *X. fastidiosa* in field-collected individuals of some species, strongly suggest that that they are involved in spread of this pathogen in olive orchards. Some of the vector species are likely more important, due to their higher population and higher levels of natural infectivity.

Keywords: Olive quick decline syndrome, key vectors, epidemiology

Support: Horizon 2020 (XF Actors 727987), Fapesp (Proc. 2016/02176-7), Capes and CNPq (Proc. 310554/2016-0)."

New insights on *Xylella fastidiosa* subsp. pauca vector transmission to olive plants

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The spittlebug *Philaenus spumarius* L. is known to be the predominant vector of *Xylella fastidiosa* Wells et al. (Xf) to olive trees in the Apulia Region of Italy. However, factors influencing Xf transmission by *P. spumarius* have not been studied and, consequently, the epidemiology of Xf-associated emerging diseases cannot be properly understood. Here we investigated 1) the kinetics of the bacterial persistence and multiplication, along with transmission efficiency by the vector, and 2) the spread rate of Xf among olive plants in summer and autumn. For the first objective, olive-to-olive transmission efficiency, Xf retention and multiplication in *P. spumarius* adults were tested in four separate assays carried out in 2017-2018 in different seasons. For the second objective, experimental assays in microcosms were carried out in the same periods to assess influence of i) IAP duration, ii) climatic conditions (semi-field vs controlled), and iii) season on the spread of Xf infection within an olive seedling population. The results show that i) *P. spumarius* is a competent Xf vector to olive throughout the season and adult life ii) bacterial load in the vector foregut reaches a plateau after 2-3 weeks from acquisition iii) different climatic conditions and period of the year may result in significant differences in transmission rates and iv) differential survival of vectors — influenced by insect age, season and climatic conditions — may affect the spread of Xf in olive plants. In summary, this work describes the influence of seasonal and environmental factors on the transmission characteristics of Xf by *P. spumarius*. Such transmission dynamics may explain epidemic patterns in Apulian olive agroecosystem. The transmission parameters obtained in this study can be used in modelling the pathogen spread, by explicitly incorporating the effect of insect vectors, with the aim of designing effective control and prevention measures.

Keywords: vector bacterial load, transmission efficiency, olive-to-olive spread rate

On the importance of multidisciplinary studies on insect vectors to better understand vector-borne plant diseases

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Insect vectors are key actors of the *Xylella fastidiosa* (Xf) pathosystem. Studies on their host plants, population dynamics and natural enemies as well as large-scale screening of populations for the presence of Xf are essential to understand and control the bacterium spread across agro-ecosystems. In this communication, we will summarize the studies conducted in Corsica since 2017 using field observations, molecular biology, statistical modeling and species distribution modelling. We will elaborate on results transferable to other European areas and, by contrast, on results indicating situations where a case-by-case management strategy appears more appropriate. We will report on the ecological factors driving the abundance of Philaenus spumarius (Ps), which is mostly associated with *Cistus monspeliensis* and shows preference for cool but relatively dry conditions. In corsican agrosystems, Ps is not the only vector found on crop foliage and other insects could play a role for disease transmission. Managing *C. monspeliensis* in the vicinity of agricultural areas may nevertheless constitute a component of prophylaxis of Xf. We will also report on an egg parasitoid of Ps occurring throughout Europe that constitutes a potential control agent to be further evaluated. Finally, we will present the results of a massive screening of vectors conducted to assess the spatio-temporal prevalence of Xf. Results suggest that Xf introduction in Corsica largely predates its detection in 2015 and we hypothesize that ecological resilience of Corsican ecosystems could be possibly linked to plant diversity and lack of monoculture farming. Deciphering and managing the epidemiology of vector-borne plant diseases require multidisciplinary approaches and a thorough understanding of insect vector ecology in addition to the analysis of plant-pathogen interaction.

Impact of low susceptible and resistant host plants on the transmission of *Xylella fastidiosa* subsp. pauca ST53 by *Philaenus spumarius* (Hemiptera: Aphrophoridae)

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In the context of the severe epidemic spread of *Xylella fastidiosa* subsp. pauca ST53 (Xfp) in the Apulia Region (southern Italy), there is a need to urgently rethink cropping system for restoring agricultural productions and landscapes. Efforts are devoted to identifying species immune to Xfp (as alternative crops), and to assess the impact of host species showing low susceptibility/resistance to the infections. To this end, we performed transmission experiments from olives and almond, to assess vector acquisition and transmission from host plants showing different susceptibility and low bacterial population size (<105 CFU/ml). We compared vector acquisition/transmission using susceptible and resistant olive cultivars as source of the bacterium: "Cellina di Nardò" (highly susceptible), "Kalamata" (susceptible), "Leccino" and "FS17" (resistant). We also included in the experiments almond trees, characterized by low prevalence and impact of Xfp-infections. Acquisition was done by caging 50-100 Xfp-free specimens of Philaenus spumarius on branches selected on field grown trees. After 3-4 days of acquisition, insects were transferred on healthy olive and periwinkle plants. Molecular diagnostic tests clearly showed differential acquisition rates (% of positive insects) according to the source plants: 21.7% for "Cellina di Nardò", 8.9% for "Kalamata", <1.8% for "Leccino" and "FS17", and 2.5% for almond. Transmission rates to olive seedlings positively correlated with the acquisition: 18.6% with insects recovered from "Cellina di Nardò", 7.0% from "Kalamata", 1.2% from "FS17", 3.4% on almond, while no transmission was recorded when insects were caged on "Leccino". Using recipient periwinkle plants, the transmission rates were: 26% with insects from "Cellina di Nardò", 5% from "Kalamata", while no transmission occurred with insects fed on "Leccino", "FS17" and almond. These data suggest that replacement of high susceptible crops with host plants harboring limited bacterial population size may substantially contribute to suppress the spread rate and the impact of the disease in the infected areas.

Vibrational-disruption of the feeding behavior of a plant pathogen vector

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Within *Auchenorrhyncha*, vibrational signals underlie interactions with conspecific and environment, and can be used to disrupt relevant behaviors. Vibrational interference with vector behaviors associated to host plant recognition and feeding could enable development of innovative management strategies. The main European vector of the fastidious bacterium *Xylella fastidiosa* is *Philaenus spumarius*, which uses vibrations to communicate. In our work, a vibrational stimulus was designed based on a *P. spumarius* signal and was transmitted over a suitable host plant (sunflower). The signal significantly reduced the probing and feeding behavior of the insect vector, which is a xylem-feeder. Specifically, ca. 30% of the treated individuals did not attempt probing, while the feeding activity of probing insects was affected by the vibrational signal. Accordingly, a sex-independent reduction in ingestion of the xylem sap of ca. 67% compared to the control was recorded. The possible implications of vibrational vector behavior interferences on the epidemiology of *X. fastidiosa* and future research needs are discussed, including the possibility of applying this technique to olive plants.

Keywords: biotremology-EPG-behavioral interference

Host plant selection by *Philaenus spumarius*: using ground covers as trap crops

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The recent introduction and settlement of the vector-borne pathogen *Xylella fastidiosa* in Europe is claiming the development of long-term containment procedures that allow controlling the disease together with a sustainable management of the crops. Nowadays, minimizing vector populations seems to be the better way to contain the disease in time. Adults of *P. spumarius* are difficult to manage because of their high mobility and polyphagy, thus control measures should focus on the more sedentary juvenile instars together with the egg phase. This study aims to search for host plant species that could act as trap or repellent plants for nymphs of *P. spumarius* that could be used as cover crops. Furthermore, the plants should be selected on the basis of *P. spumarius* oviposition preference. Firstly, choice and non-choice assays were conducted under semi-field conditions to assess the effect of selected plant species on the preference and mortality of *P. spumarius* nymphs. Ten plant species, belonging to five botanical families commonly used in the ground cover of olive and other X. fastidiosa susceptible woody crops, were selected. Our results suggest that *Diplotaxis tenuifolia* (Brassicaceae) could be used to repel the nymphs whilst *Sinapis alba* (Brassicaceae) can act as a trap crop according to the mortality and the colonization rate obtained. By contrast, *Taraxacum officinale* (Asteraceae) showed one of the highest colonization rate together with a low mortality of the nymphs. Considering these results, a second-choice assay was conducted to study the oviposition preference of *P. spumarius* females. The results showed that *T. officinale* and *Sonchus oleraceus* were the preferred hosts for oviposition of *P. spumarius*. Thus, these two plant species should be avoided as part of ground covers to be used in olive groves and other *X. fastidiosa*-susceptible crops.

Defining a set of integrated tools recommended for IPM strategy to control spittlebugs

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In the last 5 years several trials have been developed under field and semifield conditions in Apulia (southern Italy) to test the effectiveness of different strategies to control the populations of *Philaenus spumarius* (Ps) and *Neophilaenus ca*mpestris (Nc) in olive groves. Trials were optimized based on the outcomes (biology, host preference and population dynamics) of a multiyear monitoring program. It is well estabhlished that mechanical interventions for removing the ground vegetation in spring, is one of the best and environmentally sustainable strategies against juveniles. However, we demonstrated that the timing for these interventions is critical to get high suppression efficiency and should be defined locally based on the microclimatic conditions. Nevertheless, a wide range of alternative strategies have been tested: mulching, pyroherbicides, applications of herbicides/insecticides, replacement of the natural ground vegetation, reaching in some cases mortality rates close to 100%. Sowing gramineous (negatively selected by Ps) can contribute to reduce Ps nymphs but not Nc. Among insecticides, neonicotinoids and pyrethoids showed the highest efficacy in the control of juveniles of both species, while other products (i.e. buprofenzin , orange essential oil, kaolin and zeolite) contributed to reduce the nymphs population but showed lower effectiveness. With regard to adults, insecticides are so far the only practical option available. Neonicotinoids and pyrethroids showed the highest efficacy and persistence up to 15-20 and 20-25 days after treatment, respectively. High values of mortality and persistence were obtainded in trials where cyantraniliprole was used. Organophosphate insecticides yielded lower mortality rates or inconsistent results. Good knockdown effect was recorded using spinosad, spinetoram, abamectina and orange essential oil, with a persistence up to 7 days for spinetoram, but with no-persistence in other insecticides tested. Other insecticides, i.e spirotetramat, flonicamid, pymetrozine, buprofenzin, natural pyrethrin and azadirachtin, did not cause significant mortality compared to the untreated controls. As for the juveniles, investigations on the population dynamics in olive groves and other crops, allowed to identify the best timing for insecticides applications. All togheter, the data gathered from the different control strategies tested allowed to define a set of recommended interventions for reducing juvenile and adult spittlebugs.

DAY 2 – Tuesday 27 APRIL 2021 - 17.40-19.30

SESSION 3: ***Control of the bacterium in the host plants***	
Chairs: Blanca B. Landa (CSIC-IAS) and Pasquale Saldarelli (CNR-IPSP)	
17.40-17.50	Introduction to the session
17.50-18.05	Isolation and characterization of bacteriophages against *Xylella fastidiosa* ***Maria L. Domingo-Calap,*** *Empresa de Transformación Agraria (Tragsa), Delegación de Valencia, - Centro de Protección Vegetal y Biotecnología. Instituto Valenciano de Investigaciones Agrarias (IVIA), Valencia (ES)*
18.05-18.20	Exploring endophytic microbial communities to identify potential biocontrol agents against *Xylella fastidiosa* strain 'De Donno' ***Massimiliano Morelli****, Institute for Sustainable Plant Protection CNR, Bari (IT)*
18.20-18.30	Q&A
18.30-18.45	Screening of natural and eco-friendly compound for their antimicrobial activity against *Xylella fastidiosa* ***Carmine Del Grosso****, Department of Agricultural, Environmental and Food Sciences University of Molise (IT)*
18.45-19.00	Signals in pathogen and host sensing: free fatty acid and oxylipins ***Valeria Scala****, CREA, Research Centre for Plant Protection and Certification, Roma (IT)*
19.00-19.10	Q&A
19.10-19.30	**Conclusions: Micheal Maixner, Alberto Fereres and Blanca B. Landa**

Isolation and characterization of bacteriophages against *Xylella fastidiosa*

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Bacteriophage viruses are a promising tool for preventing and treating bacterial infections. In this sense, phage therapy is an eco-sustainable approach that has the potential to be used as either an alternative or a complement to other strategies for the management of *Xylella fastidiosa* diseases. The main objective of this work was the search, isolation, selection and characterization of bacteriophages against this phytopathogenic bacterium. Due to the fastidious and slow growth of *X. fastidiosa* on solid culture medium, we selected strains of phylogenetically-related *Xanthomonas* species as surrogate hosts for phage hunting in a first approach. Plant host, water and soil samples from the *X. fastidiosa* outbreaks in Balearic Islands and Alicante, as well as wastewater samples from different locations in the province of Valencia, were screened for the presence of phages able to form plaques on bacterial lawns of *Xanthomonas* spp. strains by using the overlay method. A total of 22 bacteriophages were isolated, all of them from sewage: 14 infecting the strain IVIA 1317-1a of *Xanthomonas arboricola* pv. juglandis and 8 infecting the strain CECT 914 of *X. axonopodis* pv. phaseoli. Bacteriophages were isolated, purified, and amplified. Their lytic activity was tested against more than 25 strains of *Xanthomonas* spp., and the results suggest differences in the host infection range. Ongoing experiments running to evaluate the effect of the 22 isolated bacteriophages against different strains of *X. fastidiosa* in liquid medium indicate that at least five phages are able to reduce the growth of the strains, in some cases even to total inhibition. All bacteriophages are currently being characterized phenotypically and genomically, and will be compared with previously described phages.

Exploring endophytic microbial communities to identify potential biocontrol agents against *Xylella fastidiosa* strain 'De Donno'

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The compelling search for a strategy to tackle *Xylella fastidiosa* strain 'De Donno' and the Olive Quick Decline Syndrome (OQDS) it causes in olive trees, led us to explore the possibility to identify potential biocontrol agents among the microbial communities naturally inhabiting xylem vessels of infected trees. To this aim, we pursued a multiapproach study, intended to provide unprecedented information on the composition and the possible role of olive endophytic microorganisms in mitigating the effects of *X. fastidiosa* infections. In first instance, a Whole Metagenome Shotgun Sequencing (WMSS) coupled with 16S/ITS rRNA gene sequencing was carried out on field-grown plants of the susceptible and resistant olive cvs. 'Kalamata' and 'FS17', respectively, at two different stages of the disease progress. Characterization of bacterial and fungal communities based on this approach revealed that *X. fastidiosa* had a prominent role in the ecological niche it occupied and significantly lowered the microbial diversity. Although this effect was less evident in cv. 'FS17', collectively our data indicated that host cultivar had a marginal impact on the microbiome structure, and host resistance traits could not be associated with the presence of a single microbial taxon or specific consortia. In an effort to identify potential naturally-occurring *X. fastidiosa* antagonists we shifted our sights to the culturable fraction of bacterial communities isolated from trees belonging to either resistant ('Leccino', 'FS17') or susceptible ('Kalamata') cultivars, harboring low population sizes of X. fastidiosa and showing no or mild symptoms of OQDS. Following biological and molecular characterization of yielded isolates several different species, representative of the genera *Sphingomonas*, *Methylobacterium*, *Micrococcus* and *Curtobacterium* were identified. However, when tested in antagonistic assays against *X. fastidiosa*, none of them proved to be effective. Interestingly, when we included in our study different *Bacillus* strains previously isolated from other sources, significant antagonistic effects were detected.

Keywords: microbiome; endophytes; biological control

Screening of natural and eco-friendly compound for their antimicrobial activity against *Xylella fastidiosa*

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Xylella fastidiosa (Xf) is one of the most dangerous phytopathogenic bacteria capable of infecting a large number of host plants. In the last decade, several outbreaks have been discovered in Europe and the most serious are epidemics due to *X. fastidiosa* pauca (Xfp), the causal agent of Olive Quick Decline Syndrome (OQDS) devastating olives orchards in southern Italy. Despite numerous efforts in evaluating the efficacy of plant protection products and other compounds with potential activity against the pathogen, satisfactory results have not been achieved so far. Given the continuous expansion of the so called "infected demarcated area" in Apulia, i.e. the area where the pathogen is not eradicable and only containment measures are applied, strategies are needed to mitigate the impact and rebuild agriculture and the devastated landscape. In this scenario, we are evaluating against different Xf strains and subspecies the antibacterial activity of novel eco-friendly products and compounds, like a seaweed and plant polyphenols mixture (SWP), others polyphenolic biomolecules (PB) and plant systemic zinc and copper-based formulates (Zn-F and Cu-F). In vitro experiments, determining the minimum inhibitory concentration (MIC) of each product, evidenced a dose-dependent and a broad-spectrum antibacterial activity of the tested products. To further evaluate the potential exploitation of the novel antibacterial products, in vivo experiments are underway on potted olive plants kept under controlled conditions. Olive plants treated with SWP, Zn-F or Cu-F, or untreated as control, were inoculated by grafting Xfp-infected scions from susceptible and resistant olive cultivars. Periodic surveys and diagnostic tests are in progress to monitor plant growth, disease severity and host-colonization by Xfp. Investigations will also begin to elucidate the possible mechanisms of action involved in the antibacterial activity and on effect of the novel products on the plant.

Keywords: antimicrobial actvity, polyphenols, *Xylella fastidiosa*, minimum inhibitory concentration

Signals in pathogen and host sensing: free fatty acid and oxylipins

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Lipids play important roles at various stages of host–pathogen interactions, determine pathogens virulence, modulate plant defenses and might also function as modulators of several pathways in cell-to-cell communication. Free Fatty Acids (FFA) oxidated by enzymes [e.g., lipoxygenases (LOXs) and dioxygenases (DOXs)] form oxylipins, that have been extensively studied in plant–pathogen interaction. The oxylipins show a structural similarity among plant, fungal and bacterial prompting the hypothesis that they are important in cross-kingdom communication. We presented here the results within European XF-ACTORS and Italian SALAVOLIVI and OLIDIXIIT projects. The studies were carried out with *Xylella fastidiosa* subsp. pauca (Xfp) grown in vitro amending or not different lipids entities to characterize the bacterial lipidome. The results support the hypothesis that FFA and oxylipins change in the different Xfp lifestyle and are crucial to modulate the pathogen lifestyle. Further we analysed the lipidome of *Nicotiana tabacum* and *Olea europaea*, artificially and naturally infected (Olive Quick Decline Syndrome - OQDS) with Xfp, respectively. LC-TOF and LC-MS/MS analysis pinpointed that the FFA and the oxylipins derived by the oleic, linoleic and linolenic acid are differently accumulated in infected plants versus non infected and some lipid entities are hallmarking of infection. Basing on the knowledge of oxylipins as cellular signals in different pathosystems, we can assume that lipids can act as signals for reshaping the lifestyle of the contenders and sometimes determining the fate of the challenge. These studies demonstrate, for the first time in a phytopathogenic bacteria, that the LOX- and DOX-oxylipins can influence the bacterial "status" and are differently accumulated in infected versus non infected plants. More efforts should be conducted to unveil if this lipid entities pave the way to *Xylella* pathogenicity or simply facilitate it. These hallmarks can be employed as markers of OQDS and/or for developing new control strategies.

Keywords: molecular signals, lipids, oxylipins

DAY 3 – Wednesday 28 APRIL 2021 - 14.30-19.00

SESSION 4: ***Implementation and harmonization of diagnostic protocols, surveillance and modelling***	
Chairs: Pieter Beck (JRC) and Françoise Petter (EPPO)	
14.30-14.40	Introduction to the session
14.40-14.55	Using hyperspectral imagery and a multi-stage machine learning algorithm to distinguish infection symptoms caused by two xylem-limited pathogens ***Tomás Poblete****, School of Agriculture and Food (SAF-FVAS) and Faculty of Engineering and Information Technology (IE-FEIT), University of Melbourne (AU)*
14.55-15.10	Integrating an epidemic spread model with remote sensing for *Xylella fastidiosa* detection ***Carlos Camino****, European Commission (EC), Joint Research Centre (JRC), Ispra (IT)*
15.10-15.20	Q&A
15.20-15.35	Variations in winter temperatures over decades: the underside of a French *Xylella fastidiosa* story ***Samuel Soubeyrand****, INRAE, BioSP, Avignon (FR)*
15.35-15.50	Improving early detection surveillance for *Xylella fastidiosa* in Apulia ***Alexander Mastin****, University of Salford, Salford (UK)*
15.50-16.00	Q&A
16.00-16.15	Effects of dispersal barriers in the demarcated area in Alicante, Spain, for *Xylella fastidiosa.* A non-stationary modelling approach. ***Martina Cendoya****, Instituto Valenciano de Investigaciones Agrarias, Valencia (ES)*
16.15-16.30	Developing a spatial epidemiological model to estimate *Xylella fastidiosa* dispersal and spread ***Daniel S. Chapman****, University of Stirling, Stirling (UK)*
16.30-16.40	Q&A
16.40-17.00	**Coffee break**
17.00-17.15	Spatial and temporal evolution of *Xylella fastidiosa* in the canopy of Leccino and Ogliarola olive cvs in Apulia (Italy) ***Anna Maria D'Onghia****, CIHEAM – Mediterranean Agronomic Institute of Bari (IT)*
17.15-17.30	Interlaboratory comparison of molecular methods for the detection of *Xylella fastidiosa* in plant and insects ***Giuliana Loconsole****, Institute for Sustainable Plant Protection, CNR, Bari (IT)*
17.30-17.40	Q&A
17.40-17.55	How did XF-ACTORS contribute to the work of NPPOs ***Françoise Petter****, EPPO, Paris (FR)*
17.55-18.10	Scientific contributions of XF-ACTORS to the EU plant health regime ***Antonio Vicent****, Instituto Valenciano de Investigaciones Agrarias, Valencia (ES)*
18.10-18.25	*Xylella fastidiosa*, the social and media impact in plant health management ***Angelo Di Mambro****, Informatore Agrario*
18.25-18.40	Q&A
18.40-19.00	**Closure of the XF-ACTORS final meeting**

Using hyperspectral imagery and a multi-stage machine learning algorithm to distinguish infection symptoms caused by two xylem-limited pathogens

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Two major threats affecting olives groves are the bacterium *Xylella fastidiosa* (Xf) and the soil borne fungus *Verticillium dahliae* (Vd) Kleb. Both pathogens block the water flow along the xylem vessels, triggering similar physiological symptoms which are also easily confounded with water-stress induced responses. Previous remote sensing studies have attempted to independently detect Xf and Vd infections using hyperspectral and thermal imagery, but the potential discrimination between both infections has not been addressed yet. In this study, a multistage machine learning (ML) methodology is proposed to detect and discriminate the spectral changes caused by Xf and Vd infection. The multi-stage ML approach assessed spectral plant traits retrieved by physical model inversion such as leaf pigment content (carotenoids, Cx+c, chlorophyll a+b, Ca+b and anthocyanins, Anth.), canopy structural parameters (leaf area index, LAI and the leaf inclination distribution function, LIDFa), solar-induced fluorescence (SIF@760), the thermal-based Crop Water Stress Index (CWSI) and narrow-band hyperspectral indices. On each stage, key indicators were used to i) detect infection by each of the two pathogens accounting for the importance of the spectral traits, ii) reduce the detection uncertainties due to the confounding physiological responses triggered by water stress, and iii) discriminate between Vd and Xf infections. Results showed that SIF@760, Anth. and the Normalized Phaeophytinization Index (NPQI) were the most relevant traits to identify trees showing symptoms confounded with water stress. Using a Vd+Xf cross-infected balanced dataset the multi-stage ML approach yielded overall accuracies that exceeded 92% (kappa=0.8). The most sensitive spectral plant-trait indicators to discriminate between both pathogen infections will be discussed, describing operational aspects for the large-scale monitoring of olive orchards in geographic regions potentially affected by both pathogens.

Keyword: Hyperspectral, thermal, machine learning, disease-differentiation

Integrating an epidemic spread model with remote sensing for *Xylella fastidiosa* detection

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Xylella fastidiosa (Xf) causes plant diseases that lead to massive economic losses in agricultural crops, making it one of the pathogens of greatest concern to agriculture nowadays. Detecting Xf at early stages of infection is crucial to prevent and manage outbreaks of this vector-borne bacterium. Recent remote sensing (RS) studies at different scales have shown that Xf-infected olive trees have distinct spectral features in the visible and infrared regions (VNIR). However, RS-based forecasting of Xf outbreaks requires tools that account for their spatiotemporal dynamics. Here, we show how coupling a spatial Xf-spread model with the probability of Xf-infection predicted by an RS-driven modeling algorithm based on a Support Vector Machine (RS-SVM) helps detecting the spatial Xf distribution in a landscape. To optimize such model, we investigated which RS plant traits (i.e., pigments, structural or leaf protein content) derived from high-resolution hyperspectral imagery and biophysical modelling are most responsive to Xf infection and damage. For that, we combined a field campaign in almond orchards in Alicante province (Spain) affected by Xf (n=1,426 trees), with an airborne campaign over the same area to acquire high-resolution thermal and hyperspectral images in the visible-near-infrared (400-850 nm) and short-wave infrared regions (SWIR, 950-1700 nm). We found that coupling the epidemic spread model and the RS-based model increased accuracy by around 5% (OA = 80%, kappa = 0.48 and AUC = 0.81); compared to the best performing RS-SVM model (OA = 75%; kappa = 0.50) that included as predictors leaf protein content, nitrogen indices (NIs), fluorescence and a thermal indicator, alongside pigments and structural parameters. The parameters with the greatest explanatory power of the RS model were leaf protein content together with NI (28%), followed by chlorophyll (22%), structural parameters (LAI and LIDFa), and chlorophyll indicators of photosynthetic efficiency. In the subset of almond trees where the presence of Xf was tested by qPCR (n=318 tress), the combined RS-spread model yielded the best performance (OA of 71% and kappa = 0.33). Conversely, the best-performing RS-SVM model and visual inspections produced OA and kappa values of 65% and 0.31, respectively. This study shows for the first time the potential of combining spatial epidemiological models and remote sensing to monitor Xf-disease distribution in almond trees.

Keywords: Hyperspectral, thermal, *Xylella fastidiosa*, nutritional, spread model

Variations in winter temperatures over decades: the underside of a French XF story

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Xylella fastidiosa was in situ detected in France in 2015 (in Corsica and Provence-Alpes-Côte d'Azur) but several studies, grounded on phylogenetic and population-dynamics models, trace the emergence of *Xylella* in Corsica from the 50's to the 80's (at least with regard to the strains observed since 2015). At this temporal scale (i.e., at the scale of the decades separating the emergence of *Xylella* and its detection), *Xylella* and its vector(s) encountered definitively diverse climatic conditions, because of both weather variations and climate change. They especially encountered varying winter temperatures, which are classically used to build proxies of *Xylella* presence or severity. Combining knowledge from the literature, surveillance data and a parsimonious dynamic model that explicitly takes into account the spatio-temporal variation in winter temperatures, I will (i) present possible past propagation scenarios up to the detection of *Xylella*, (ii) disentangle the impact of annual variation in winter temperatures in these scenarios, (iii) assess the impact of surveillance and control measures in the contemporary period, and (iii) draw forecasts of the sanitary situation during the next decades grounded on different representative pathways of CO2 concentration. Such an analysis, based on a dynamic vision of the spatial distribution of *Xylella* over broad temporal horizons, could become an interesting tool to informing the choices and the timing of strategies for eradication, containment, living with, etc.

Keywords: population dynamics, epidemic scenarios, spatio-temporel modeling

Improving early detection surveillance for *Xylella fastidiosa* in Apulia

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The emergence of *Xylella fastidiosa* in Europe has highlighted the importance of surveillance for protection of host plants against exotic pathogens. However, surveillance for such pathogens is resource intensive and must therefore be efficient and sustainable. Using the example of the Apulian outbreak, we consider here how best to conduct surveillance in pest-free areas which are at a high risk of infection due to spread from adjacent outbreaks. To do this, we consider where inspection and survey sites should be, which diagnostic test to use during surveillance, and whether to sample hosts or insect vectors. We identify the optimal locations of surveillance sites by linking together an epidemiological model of pathogen spread and a statistically-informed sampling model with a computational optimisation routine. This approach allows us to demonstrate the considerable differences in survey deployment in order to maximise the number of infected hosts detected compared with maximising the overall probability of detection. We go on to consider the impact of the ""detection lag"" before infected hosts become detectable on both the detection probability and the expected prevalence of infection at the time of first detection. Finally, we consider whether sampling of hosts or sampling of vectors would be expected to facilitate earlier detection, and discuss some of the challenges associated with these strategies. As well as providing specific recommendations for improving surveillance activities for *X. fastidiosa* in Apulia, our methods are generic and can be applied in other areas and for a range of pests and pathogens.

Keywords: surveillance; mathematical modelling; detection

Effects of dispersal barriers in the demarcated area in Alicante, Spain, for *Xylella fastidiosa*. A non-stationary modelling approach

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Spatial models for plant diseases often assume isotropy and stationarity, implying that the spatial dependence is direction invariant and uniform through the study area. However, these assumptions are violated when dispersal barriers are present in the form of geographical features or disease control interventions. The main objective here was to evaluate the influence of different types of barriers in the distribution of *Xylella fastidiosa* (Xf) in the demarcated area in Alicante, Spain. Occurrence data of Xf from the official surveys in 2018 were analysed with four spatial Bayesian hierarchical models: i) stationary model representing a scenario without control interventions or geographical features affecting Xf spread; ii) model without control interventions but with mountains as physical barriers for Xf spread; iii) model with a continuous or iv) discontinuous cordon sanitaire surrounding the infested area to contain the pathogen. The methodology assumes that barriers are totally impermeable, so they should be interpreted as areas without host plants and in which it is not possible for infected vectors or propagating plant material to pass. The spatial range for the stationary model indicated that host plants being closer than 4.19 km 95%CI (3.01, 5.84) to an infected plant would be at risk for Xf. This distance can be used to define the buffer zone around the infested area in Alicante. The probability of Xf presence in the breaks of the cordon sanitaire was related with the availability of data, being higher in areas of low sampling intensity. These results may assist authorities to prioritize the areas for surveillance and implementation of control measures. To our knowledge, this study is the first applying non-stationary models with barriers in the context of plant health. Nevertheless, new modelling methods need to be developed to accommodate barriers with different levels of permeability.

Keywords: Containment, barriers, non-stationary models

Developing a spatial epidemiological model to estimate *Xylella fastidiosa* dispersal and spread

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Understanding the dispersal of *Xylella fastidiosa* is essential for effective management of the disease. In Puglia, Italy, surveillance is focused on buffer and containment zones established at the edge of the infected region with the aim of containing further spread. Success of this strategy will strongly depend on whether these zones are wide enough to form a barrier to long distance dispersal of the bacterium. In this presentation, I will describe our progress towards estimating the dispersal range of *Xylella* in Puglia using a generic spatial epidemiological model adapted to the biology of the pathosystem. The model simulates the spread of the disease across a heterogeneous landscape depending on the location and timing of introduction, the distribution of host plants, the rate of infection growth in infected olive groves and both short and long distance dispersal. Long distance dispersal seems to be a crucial feature of the *Xylella* epidemic, causing rapid spread of the disease over large areas but in an unpredictable manner. To calibrate the model, we used Approximate Bayesian Computation to compare model simulations to *Xylella* surveillance data and remote sensing of severe damage. This allows us to contrast a simple spread scenario with more complex scenarios such as anisotropic dispersal in the direction of prevailing winds and spatial variation in disease transmission. In doing so we characterise the spread and estimate the year of introduction. Finally, I will discuss potential for using the model to simulate management strategies and new outbreaks in other regions, using the UK as a case study.

Keywords: Modelling, spread, dispersal

Spatial and temporal evolution of *Xylella fastidiosa* in the canopy of Leccino and Ogliarola olive cvs in Apulia (Italy)

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In the framework of the official monitoring programme of *X. fastidiosa* in the Apulian buffer zone and pest-free area, sampling and testing were carried out on olive trees, as primary host species of De Donno strain. The spatial and temporal progression of the infection was studied in the canopy of asymptomatic or low-symptomatic trees of tolerant Leccino and susceptible Ogliarola cvs from 2 infected olive groves. A total of 640 samples/cv from 2 canopy levels (high and low) were analyzed in 4 collection times (June, September, December, March) testing 2 matrices (xylem tissue from twig and from mature leaf with petiole) for qualitative and quantitative analyses of the infection (ELISA, DTBIA, qPCR). The infection rate in the high level of the canopy was higher than in the low level in Ogliarola cv, while the difference between the two levels was less evident in Leccino cv. With respect to serological testing, qPCR showed the best results with both matrices in all testing times except in September with leaves. Twig xylem tissue was also the best matrix for serological tests. However, ELISA and DTBIA showed the lowest infection rate in September using both matrices. The trend in infection rate was different between the 2 cvs over the entire testing period. It remained in a range between 20% and 40% in Leccino cv, while it showed an exponential increase in Ogliarola cv where it reached even 100% in the high part of the canopy. Twig xylem tissue was also the best matrix for pathogen quantification in both cvs, more evident in the high level of the canopy. For both cvs, pathogen concentration increased in the winter period and decreased in the summer period. The decrease continued until December only in Leccino cv with the twig matrix. Based on these results an improved sampling and testing protocol was proposed for monitoring *X. fastidiosa* in olive trees.

Keywords: sampling, DTBIA, ELISA, qPCR, De Donno strain

Interlaboratory comparison of molecular methods for the detection of *Xylella fastidiosa* in plant and insects

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In 2020, an Interlaboratory Comparison (IC) was organized for the identification of *Xylella fastidiosa* (Xf) by qPCR in plant and insect samples. In the framework of this IC a test performance study (TPS) and a proficiency test (PT) involving 16 and 14 laboratories, respectively, were organized. All participating laboratories processed the samples (crude sap containing inactivated bacterial cells) from the DNA extraction step by comparing different procedures.

The TPS aimed to compare the performance of different DNA extraction procedures, whereas, the PT assessed the proficiency of diagnostic laboratories carrying out Xf diagnosis.

The values of accuracy, repeatability and reproducibility recorded for the three DNA extraction procedures ranged from 98.52% to 99.30% when testing plant samples, and from 94.07% to 98.75% for insect samples, with a the detection limit of approx. 10^2 CFU/ml. Overall, the data gathered confirmed the robustness of the procedures previously validated (CTAB, DNeasy mericon Food kit - Qiagen), and allowed to extend the panel of DNA extraction procedures currently available for processing the wide range of plant matrices to be checked for Xf (i.e Maxwell® RSC PureFood GMO and Authentication Kit-Promega). Both commercial kits tested, combined with automatized platforms, yielded better performance than the manual CTAB-based procedure.

All laboratories participating to the PT proved to be highly proficient (values close to 100% for all performance criteria), regardless the procedure of extraction, the qPCR master mix and the amplification conditions used. However, some of the extraction methods used in few laboratories produced lower yield and quality of the total DNA recovered, thus producing higher values of quantitation cycle in qPCR.

In conclusion, the TPS provided useful information on additional extraction procedures that can be used to efficiently detect the pathogen in different plant and insect matrices. While the PT provided hints on the efficiency of the diagnostic laboratories operating in 10 different EU/non-EU Countries.

Keywords: interlaboratory comparison, molecular tests

How did XF-ACTORS contribute to the work of NPPOs

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The European and Mediterranean Plant Protection Organization (EPPO) is a regional standard-setting organization in plant health. One of EPPO's main priorities is to prevent the introduction of dangerous pests from other parts of the world, and to limit their spread within the region should they be introduced. After the introduction of *Xylella fastidiosa* in the region, the first priority for the organization has been the revision of the Diagnostic protocol (first adopted in 2004). Standards on inspection of consignments and place of production have also been prepared. EPPO has also initiated the revision of the certification scheme for olive plants for planting, benefiting from the contacts and technical discussions made during participation in the development of the XF-ACTORS Voluntary System Preventing Pests (VSPP)EPPO also initiated the revision of the certification scheme for olive plants for planting, benefiting from the contacts and technical discussions made during participation in the development of the XF-ACTORS Voluntary System Preventing Pests (VSPP). Finally, discussions between modelers and risk managers have been organized in the framework of the project. The contribution of XF-ACTORS to the revision of these Standards will be highlighted as well as other activities conducted with XF-ACTORS partners which are of benefit to National Plant Protection Organizations.

Xylella fastidiosa, the social and media impact in plant health management

Di Mambro A

Informatore Agrario

Been following the *Xylella fastidiosa* (Xf) epidemics in Europe, focusing on the cultural, social, and media aspects. In the specific case, the emergency's public perception has been critical. The lack of social acceptance of the epidemic and of the authorities proposed measures to tackle it, severely impacted the eradication and containment program in its initial stages and long after, especially in Apulia. Concentrating on the Apulia case, I examined the articles on *Xylella fastidiosa* listed in the MedySis database (https://medisys.newsbrief.eu/medisys/homeedition/it/home.html), the takes from 2013 to 2017 (the period of most intense debate on Xf) in the archive of the Italian main news agency ANSA, as well as a personal collection of international and Italian press reports on the topic. I found that the public perception and media coverage of *Xylella fastidiosa* presented the same features of human epidemics' psychology, suggesting the opportunity of a ""one-health approach"" in considering the social and cultural aspects in epidemics both in plant and human health. Given the growing risk from invasive pests in the EU, my work aims to be a contribution to better integrate cultural and social aspects in plant health emergencies' management. I propose some basic strategies to include human sciences factors in developing and implementing sustainable pest management control and eradication programs.

Keywords: sustainable management, preparedness, one-health approach.

3rd European conference on *Xylella fastidiosa*

Building knowledge, protecting plant health

DAY 4 – Thursday 29 APRIL 2021 - 14.00-19.40 CEST

	Opening ceremony and Introduction to the event
14.00–14.15	Conference opening ***Bernhard Url****, Executive Director, European Food Safety Authority*
14.15-14.30	Introduction to the conference ***Giuseppe Stancanelli,*** *Animal and Plant Health Unit, European Food Safety Authority*
SESSION 1: ***Advances on pathogen biology, ecology and genetics*** Chairs: Ralf Koebnik (Plant Health Institute of Montpellier (PHIM), Institut de Recherche pour le Développement (IRD) (FR)) and Massimiliano Morelli (Institute for Sustainable Plant Protection (IPSP), National Research Council of Italy (IT)	
14.30-14.45	Aggressiveness of Spanish isolates of *Xylella fastidiosa* to almond cultivars ***Aina Baró****, University of Girona (ES)*
14.45-15.00	Assessing strain-level variation among type I restriction-modification systems and methylation patterns in *Xylella fastidiosa* ***Michael O'Leary****, US Department of Agriculture, Agricultural Research Service (US)*
15.00-15.05	A model and image based investigation of *Xylella fastidiosa* within host dynamics - *Young researchers' initiative* ***Nancy Walker****, Faculty of Engineering and Physical Sciences, University of Southampton (UK)*
15.05-15.20	Q&A
15.20-15.25	Short break
15.25-15.40	Distribution dynamics of *Xylella fastidiosa* within almond tree organs through different physiological stages ***Ofir Bahar****, Agricultural Research Organization – Volcani Center (IL)*
15.40-15.55	Genome rearrangements of *Xylella fastidiosa* strains in US and updates on *X. taiwanensis* ***Jianchi Chen****, US Department of Agriculture, Agricultural Research Service (US)*
15.55-16.00	Application of phage display: development of tools to fight against *Xylella fastidiosa* *- Young researchers' initiative* ***Favelin Nicolas****, Génie Enzymatique et Cellulaire (CNRS UMR 7025), Université de Technologie de Compiègne (FR)*
16.00-16.15	Q&A
16.15-16.30	The Arabidopsis immune receptor EFR confers immunity against *Xylella fastidiosa* ***Silke Robatzek****, Ludwig Maximilians Universität München (DE)*
16.30-16.45	*Xylella fastidiosa* biogeography and evolution: emerging insights and observations ***Rodrigo Almeida****, University of Berkeley (US)*
16.45-16.50	Csp1, a cold-shock protein homolog in *Xylella fastidiosa* is involved in stress response and biofilm formation - *Young researchers' initiative* *Wei Wei, US Department of Agriculture, Agricultural Research Service (US)*
16.50-17.05	Q&A
17.05-17.20	**Coffee break**

Aggressiveness of Spanish isolates of *Xylella fastidiosa* to almond cultivars

Baró A

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Almond leaf scorch is causing important crop losses in almond orchards in Spain since 2017. Although the epidemiology of *X. fastidiosa* diseases has been the object of research during many years, understanding why outbreaks in a similar climatic area and crop have different intensity is still needed. This can be attributed to differences in pathogenicity among the strains responsible of the outbreaks. In the present study, the aggressiveness of Spanish isolates of *X. fastidiosa*, representing different sequence types, was studied in almond cultivars.

The dynamics of the population levels and symptoms of a strain of *X. fastidiosa* subsp. fastidiosa showed doubling times of 2.1 to 2.5 days during the exponential growth phase, with a maximum population size around 35 dpi. Comparing the population dynamics in sap and xylem tissue after the exponential growth, a differential pattern was observed: while population levels in the xylem tissue remained stable, viable cells in sap decreased. This is in agreement with the already predicted model for *X. fastidiosa* plant colonization. Regarding disease severity, the first symptoms were observed between 20 and 60 dpi and increased over time with a maximum observed at 120 dpi when plants had one-half or more of the leaves showing marginal necrosis but still actively growing.

X. fastidiosa strains isolated from almond, cherry and grapevine were used to inoculate three almond cultivars (Avijor, Soleta and Penta). Differences in their maximum population levels and capacity to spread from contaminated plant parts to new growing shoots after pruning were observed. Additionally, dose-response relationships were performed with the almond isolates, showing different performance when inoculated in Avijor cultivar.

This study raises implications for the epidemiology of *X. fastidiosa* in almond crops, showing relatively short doubling times of the pathogen and symptoms development, as well as differential aggressiveness between strains.

Keywords: Prunus, aggressiveness, growth dynamics

Assessing strain-level variation among type I restriction-modification systems and methylation patterns in *Xylella fastidiosa*

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Xylella fastidiosa is a bacterial plant pathogen with five genetically distinct subspecies that collectively cause disease on a diverse set of host plants. Genomes of *X. fastidiosa* strains are enriched in genes encoding Restriction-Modification systems, which methylate native DNA and cut unmethylated DNA at specific sequence motifs. Type I Restriction-Modification systems function as a protein complex composed of modification (HsdM), restriction (HsdR), and specificity (HsdS) subunits. Sequence recognition of Type I Restriction-Modification systems depends on HsdS, which contains two target recognition domains (TRDs) that each recognize a 3-5 bp sequence on either side of a 4-7 bp nonspecific spacer. TRD sequences are extraordinarily variable; nucleotide and amino acid sequences of TRDs with different sequence specificities typically share little to no homology. Recombination between hsdS genes can generate new TRD domains combinations, resulting in hsdS alleles with novel sequence specificities. Analysis of genome assemblies of 117 *X. fastidiosa* strains reveals each strain contains three conserved Type I Restriction-Modification systems, while strains of subspecies multiplex and pauca contain a fourth system absent from other subspecies. Three to twenty-two unique hsdS alleles and two to fourteen unique TRD domains were identified per system. In total, at least 30 combined hsdS allele profiles were identified across *X. fastidiosa* strains. Distribution of hsdS alleles is associated with MLST classification but does not correlate with geographic origin or host of isolation. Genomic methylation profiles of twelve *X. fastidiosa* strains were derived using nanopore sequencing. Two to four methylated motifs characteristic of Type I Restriction-Modification recognition sites (e.g., bipartite) were identified per strain, suggesting some of these systems are active. Variation among bipartite motif sequences was identified within and between *X. fastidiosa* subspecies and correlates with variation among hsdS alleles, allowing for identified motifs to be tentatively assigned to specific Type I Restriction-Modification systems and hsdS alleles.

A model and image based investigation of *X. fastidiosa* within host dynamics

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Xylella fastidiosa (*X. fastidiosa*) is a bacterium that colonises internal plant vascular networks causing pathogenic effects on several commercially important crops (e.g. olives, grapes, coffee, etc.). Despite a growing research effort since the recent detection of *X. fastidiosa* in Europe, the exact processes leading to *X. fastidiosa* disease symptoms are not fully understood due to difficulties in observing internal plant structures. Our goal is to utilise models to elucidate fundamental processes that lead to olive quick decline syndrome, the disease responsible for the devastating olive decline in Apulia, Southern Italy. We endeavour to do this by developing a fundamental mathematical modelling framework describing within-host biofilm development, and the resulting water-stresses that ultimately inhibit plant functionality and development. We have begun work on developing a fluid mechanics model that invokes a polymer-physical description of *X. fastidiosa* biofilm formation dynamics. Our approach is centred on the assumption that the biofilm structure is determined by the arrangement of extracellular polysaccharide (EPS) molecules. We have also produced X-ray Computed Tomography (XCT) images of both resistant and susceptible olive cultivars to determine possible morphological differences between the vascular networks of the cultivars. Preliminary modelling results of EPS dominant biofilm formation characterise biofilm coalescence in a consistent manner with early microfluidic controlled experiments and require fewer empirical assumptions. We intend to generate model domains with the XCT images and apply our theoretical framework to simulate infection in actual vascular networks via image based modelling. We hope our work will improve the understanding of possible cultivar resistance mechanisms to enable informed breeding and effective replanting in Apulia, and that model simulations will provide insights for improving the efficiency of current sampling techniques.

Distribution dynamics of *Xylella fastidiosa* within almond tree organs through different physiological stages

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Xylella fastidiosa was identified in Israel ~5 years ago, infecting almond orchards in Northern Israel and causing almond leaf scorch (ALS) disease. Thus far, only a single sequence type (ST) was found, ST1, which in artificial inoculation experiments was found to infect almond and grape varieties. During field monitoring for ALS, we noticed that when infected almond trees flush in spring they do not show ALS symptoms and the foliage appear green and heathy. Symptoms begin to appear only ~two month later and become more evident during summer. This phenomenon was seen year after year with the same infected trees. We were therefore interested in understanding the cycle of *X. fastidiosa* during the different seasons and physiological stages of the tree and within its different organs. To address this question, we sampled leaf petioles, green stems, woody stems, roots, green fruit, dry fruit, flower buds and flowers from infected trees, through two complete seasons and tested them for the presence of *X. fastidiosa* using qPCR. We found that in spring *X. fastidiosa* is not detected in leaf petioles of infected trees, but it can be detected before symptoms appear in early summer. Before entering dormancy, the titer of *X. fastidiosa* in leaf petioles decreases, but it could still be detected. Unlike in leaf petioles, *X. fastidiosa* could reproducibly be detected in green and woody stems of infected trees throughout the year. The titer of *X. fastidiosa* in these tissues remained quite constant including during dormancy. *X. fastidiosa* was not detected in any of the other tissues tested throughout all sampling points. Our results reveal interesting information regarding the yearly cycle of *X. fastidiosa* in deciduous trees. It also provides important evidence that *X. fastidiosa* can be detected using molecular methods in infected trees before symptom appearance and even during dormancy.

Genome rearrangements of *Xylella fastidiosa* strains in US and updates on *X. taiwanensis*

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Xylella fastidiosa is a Gram-negative and nutritionally fastidious bacterial pathogen causing Pierce's disease (PD) of grapevine and other plant diseases in the USA. X. taiwanensis causes pear leaf scorch disease in Taiwan. Bacterial genomics is fundamental for biological research and pathogen management. Current research in genomic variations of *X. fastidiosa* mostly focuses on single nucleotide polymorphisms (SNPs), short sequence insertions/deletions (Indels), and copy number variations (CNVs). Structural variations (SVs), e.g. chromosomal rearrangements, was reported between *X. fastidiosa* CVC strain (subsp. pauca) and PD strain (subsp. fastidiosa). In this study, the whole genome sequence of X. f. subsp. fastidosa strain ATCC 35879 causing PD in Florida was determined using PacBio RSII format. The ATCC 35879 genome has a circular chromosome of 2,565,504 bp with 2,904 predicted protein coding genes and 55 RNA genes, and a circular plasmid of 41,753 bp. The chromosomal sequence of strain ATCC 35879 was compared to that of *X. fastidiosa* subsp. fastidiosa strain M23 causing both PD and almond leaf scorch disease in California. Genome rearrangements involving a ca. 1,200 Kbp region were detected. Genome annotations showed clusters of phage-related genes around the rearrangement junctions, suggesting the likely involvement of phage activities. Genome rearrangement events were also detected/confirmed between different subspecies of *X. fastidosa* and between *X. fastidosa* and *X. taiwanensis*. *X. taiwanensis* is the second species described under the genus *Xylella* in 2016. Little information about *X. taiwanensis* is available. DNA of a pear tree infected with *X. taiwanensis* was extracted and subjected to next generation sequencing (Illumina HiSeq). Metagenomic analysis on the sequence data detected a small plasmid of 2,124 bp. This plasmid showed > 65% similarities to plasmids from *X. fastidiosa*, *Xanthomonas axonopodis* and *Stenotrohomonas maltophilia*. Further genomic and metagenomic studies are underway.

Keywords: *Xylella* genomics, genome rearrangement, plasmids.

Application of phage display: development of tools to fight against *Xylella fastidiosa*

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Xylella fastidiosa is a phytopathogen infecting a wide array of plants, whose spread and effect on agricultural economy worry governments around the world. Despite many researches, its biology and in-planta behavior are still partially known and there is no curative treatments to date. In this study, we focus on a lipase/esterase called LesA, which is highly secreted by Xf, and also involved in the bacteria's virulence. This uncharacterized enzyme of 42 kD size, comprises a rigid 7 alpha-helixes LID domain, similar to lipases from other phytopathogens like *X. Oryzae*. We aim to find molecules able to bind LesA specifically and with a high affinity, to develop tools against Xf. Peptides able to recognize LesA could be used as a biomarker of Xf infection. Inhibition of an essential virulence factor could also delay Xf spread inside the host as well as emergence of symptoms. This delay may give time for plants to take an advantage on bacteria and defend themselves with a natural and appropriate defense response from its immunity, helping to fight against the disease. For this purpose, we use phage display technology to screen among a large bank of peptides those able to interact with our target enzyme. More than a billion of different peptides are expressed on surface of bacteriophages, but few will be selected by biopanning for their ability to bind the target. The resulting population of peptides will be tested by ELISA to quantify their specificity to LesA. Among the best candidates, we will select those with the additional ability to inhibit LesA and we will test them on various biological models (bacterial culture media, in-planta…).

Keywords: lipase, phage display, peptide

The Arabidopsis immune receptor EFR confers immunity against *Xylella fastidiosa*

Robatzek S

LMU München, Germany Rybak, R.

Plants have evolved a sophisticated, cell-autonomous innate immune system to recognize pathogens and oppose infection. Cell surface-localized pattern recognition receptors (PRRs) detect microbe-associated molecular patterns (MAMPs) and activate immune signalling. The presence/absence of PRRs encoded by a plant species, its cell type expression pattern and level are determinants of the plant's immune capacity. Our interest is to understand how the plant's immune system responds to infection by *Xylella fastidiosa*. Using *Arabidopsis* as a model host, we aim to identify PRRs and investigate pattern-triggered immunity (PTI) in vascular tissues. Wild type plants produced a prototypic reactive oxygen species (ROS) burst when challenged with living X. fastidiosa. This ROS burst was markedly reduced in mutants lacking the ELONGATION FACTOR-TU RECEPTOR (EFR), suggesting the perception of immunogenic elf peptides present in the bacterial MAMP EF-Tu. Since EF-Tu is present in outer membrane vesicles (OMVs) of X. fastidiosa, we determined the effect of OMVs on *Arabidopsis* seedling growth, which is typically inhibited by continual MAMP treatment. The growth of Arabidopsis wild type seedlings was strongly repressed in the presence of *X. fastidiosa* OMVs. No growth repression was observed in efr-1 or bak1-5, a mutant affected in the EFR co-receptor BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED RECEPTOR KINASE 1 (BAK1) involved in PTI. We then examined whether EFR modulates the success of *X. fastidiosa* infection in *Arabidopsis*. Compared to wild-type plants, efr-1 mutants supported higher bacterial loads of *X. fastidiosa*, quantified as HL gene abundance. Thus, perception of EF-Tu (and potentially derived elf peptides) by EFR is sufficient to restrict *X. fastidiosa* colonization in *Arabidopsis*. Our data identify that EFR functions as PRR in the interaction with *X. fastidiosa*, which could be explored for improving immunity in crops.

This work is supported by the European Research Council (ERC), and the German Research Foundation (DFG).

Xylella fastidiosa biogeography and evolution: emerging insights and observations

Almeida R

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The emergence of *Xylella fastidiosa* diseases in Europe and elsewhere has highlighted the limited knowledge available on *X. fastidiosa* diversity, biogeography, host specificity, and evolution. While often confined to academic realms, significant knowledge gaps in these topics have had important consequences to quarantine, eradication, and pathogen containment measures in European disease outbreaks. We will discuss recent research findings on these topics, and how they apply to ongoing epidemics. The talk will focus on *X. fastidiosa* biogeography and global dispersal pathways, what happens to pathogen populations after introductions into novel environments (gene gain and loss, recombination, mutation), and recent efforts to infer host specificity. We will also identify challenges for future research.

Csp1, A Cold-Shock Protein Homolog in *Xylella fastidiosa* Is Involved in Stress Response and Biofilm Formation

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Bacterial cold shock-domain proteins (CSPs) are conserved nucleic acid binding chaperone proteins that play important roles in adaptation to environmental changes and stressors such as cold adaptation and conditions unrelated to temperature. Csp1, a temperature-independent cold shock protein homolog, acts as a virulence factor in *Xylella fastidiosa*, a bacterial pathogen of grapevine and other economically important crops. In addition to virulence, Csp1 contributes to *X. fastidiosa* survival at low temperatures and under high salt and oxidative stress conditions, in vitro. However, little is known about the specific function(s) of Csp1 in *X. fastidiosa*, aside from general single-stranded nucleic acid binding activity. To investigate the influence of Csp1 on *X. fastidiosa* gene expression, we used Nanopore's MinION to sequence transcriptomes of wild-type *X. fastidiosa* strain Stag's Leap and a csp1 deletion mutant (Δcsp1) under standard growth conditions (28°C) and cold stress (15°C). Our analysis revealed changes in expression of several genes important for motility and biofilm formation in Δcsp1 compared to wild-type. One gene of intertest, pilA1, encodes a type IV pili subunit protein and was up-regulated in the Δcsp1 mutant. The type IV pili is required for twitching motility in *X. fastidiosa* and contributes to virulence by facilitating bacterial movement inside the host vascular tissue. Previous studies in other strains of *X. fastidiosa* showed deleting pilA1 leads to overabundance of type IV pili and increased biofilm formation. Xylem vessel occlusion caused by biofilms is one major mechanism of *X. fastidiosa* pathogenicity, so Csp1 may act as a virulence factor by influencing the expression of genes involved in biofilm formation and motility. We also observed a significant decrease in biofilm formation with the Δcsp1 mutant compared to the wild type strain in liquid media, which may be the result of increased expression of pilA1. The csp1 mutant was also less viable during long term growth compared to the wild-type strain, in vitro, further indicating Csp1 may be involved in stress response. Studies in other bacteria have identified links between stress response and biofilm formation, suggesting that Csp1 may play a role in both virulence and stress response by influencing genes important for biofilm formation.

SESSION 2: ***Understanding vectors biology and pathogen transmission*** Chairs: Astrid Cruaud (French National Institute for Agriculture, Food, and Environment (INRAE) (FR)) and Saskia Hogenhout (John Innes Centre (UK)	
17.20-17.35	The association of Salicaceae and Aphrophoridae: a potential *Xylella fastidiosa*-based threatening pathosystem for European temperate areas ***Noemi Casarin****, Université catholique de Louvain (BE)*
17.35-17.50	Accounting for the climate tolerances of *Philaenus spumarius* is crucial when assessing *Xylella fastidiosa*-related disease outbreaks risk in Europe ***Martin Godefroid****, Instituto de Ciencias Agrarias, Consejo Superior de Investigaciones Científicas (ICA-CSIC) (ES)*
17.50-18.00	Q&A
18.00-18.05	Short break
18.05-18.20	Spread of *Xylella fastidiosa* by the glassy-winged sharpshooter in the San Joaquin Valley of California ***Mark Sisterson****, US Department of Agriculture, Agricultural Research Service (US)*
18.20-18.35	Understanding the epidemiological role of the vectors of *Xylella fastidiosa* in the Balearic Islands (Spain) by long-term macrocosm and microcosm studies ***Miguel Ángel Miranda****, University of the Balearic Islands (ES)*
18.35-18.45	Q&A
18.45-18.50	Short break
18.50-19.05	Modelling the population dynamics of *Philaenus spumarius*: a fully mechanistic approach ***Gianni Gilioli****, Department of Molecular and Translational Medicine (DMMT), University of Brescia (IT)*
19.05-19.10	Transmission of *Xylella fastidiosa* subsp. *pauca* to olive trees by sharpshooters and spittlebugs common in Brazilian orchards - *Young researchers' initiative* ***Joyce Adriana Froza*** *(), College of Agriculture "Luiz de Queiroz", University of São Paulo (BR)*
19.10-19.15	DNA barcoding and assessment of the genetic diversity of the *Xylella fastidiosa* vectors in the Balearic Islands ***Sofía Delgado-Serra*** *(Young researchers' initiative), University of the Balearic Islands (ES)*
19.15-19.20	Microbial assemblages within *Philaenus spumarius* and their possible role on insect´s reproduction ***Cristina Cameirão*** *(Young researchers' initiative), Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança (PT)*
19.20-19.40	Q&A
	End of first day

The association of Salicaceae and Aphrophoridae: a potential *Xylella fastidiosa*-based threatening pathosystem for European temperate areas

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Riparian areas have been highlighted as favorable sites for *Xylella fastidiosa* spread and persistence as they are considered to be a niche for several insect vectors. To assess the risk of *X. fastidiosa* for Belgium, and more extensively for regions with a similar temperate oceanic climate, plant species from riparian environments as well as potential insect vectors associated with these plants have been studied, revealing yet undiscovered host plants. The distribution and the abundance of five potential insect vector species were evaluated on the Belgian territory. The dispersal capacity of two of them, *Philaenus spumarius* and *Aphrophora salicina*, was surveyed by mark-release-recapture and flight mill experiments. A. salicina reveals a higher flying capacity than *P. spumarius*, with an average travelling distance four times longer. While *P. spumarius* remains particularly dangerous because of its polyphagy, A. salicina is considered also a threat because of its specificity to the Salicaceae, a plant family typical of riparian zones for which some species showed in parallel studies their potential as good hosts for the bacterium. These features, combined with its dispersal capacity, make A. salicina a significant potential insect vector to be considered in temperate oceanic climate regions.

Accounting for the climate tolerances of *Philaenus spumarius* is crucial when assessing *Xylella fastidiosa*-related disease outbreaks risk in Europe

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The bacterium *Xylella fastidiosa* (Xf) is an invasive insect-borne pathogen, which causes lethal diseases to economically-important crops including olives, citrus, almonds and grapes as well as numerous forest, ornamental, and uncultivated plants. The meadow spittlebug *Philaenus spumarius L.* (1978) (Hemiptera: Aphrophoridae) is currently the main epidemiologically relevant vector of Xf involved in Mediterranean outbreaks. Most available risk assessments focus solely on the climatic tolerances of the bacterium and incompletely represented Xf epidemiology by omitting vector ecology and distribution. In the present study, we fitted bioclimatic species distribution models to depict the macroclimatic preferences of the meadow spittlebug and predict which regions of Europe are the most climatically suitable for this vector. The models predict a marked spatial variation in climatic suitability throughout the European continent for the meadow spittlebug, cool and moist regions being optimal. Noticeably, we found that severe Xf-related disease outbreaks have only occurred in Mediterranean areas predicted as highly suitable for P. spumarius. Conversely, no severe Xf-related disease outbreak has ever occurred in warm and dry areas of the Mediterranean basin predicted as little suitable for the vector, even though some of these regions have ideal climate conditions for multiplication of the bacterium in plants. This pattern strongly suggests that climate tolerances of P. spumarius should always be accounted for in further risk assessments of Xf outbreaks. These results have crucial practical application since the geographic extent predicted as little suitable for this vector encompasses some of the most economically-important agricultural regions of the Mediterranean basin (e.g. the first olive-producing region of the world in the Iberian peninsula). The climate suitability maps for P. spumarius are crucial for the design of current and future strategies to control Xf in Europe and call for a complete reassessment of potential Xf impact to European agriculture.

Spread of *X. fastidiosa* by the glassy-winged sharpshooter in the San Joaquin Valley of California

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The glassy-winged sharpshooter is an invasive vector of *Xylella fastidiosa* that arrived in California in the late 1980's. Pierce's disease epidemics in the Temecula Valley in the late 1990's and in the southern San Joaquin Valley in the early 2000's were associated with high glassy-winged sharpshooter abundance. In response, an area-wide glassy-winged sharpshooter suppression program was initiated that successfully reduced glassy-winged sharpshooter populations for nearly a decade. However, beginning in 2009 glassy-winged sharpshooter populations in the southern San Joaquin Valley began increasing and as a result another epidemic occurred. A field study was conducted to provide quantitative estimates of the effects of time of year on prevalence of X. fastidiosa-positive sharpshooters and detection of *X. fastidiosa* in chronically infected grapevines. Vineyards were sampled to assess the abundance of glassy-winged sharpshooters, with all sharpshooters subjected to qPCR to determine if *X. fastidiosa* was present in insect mouthparts. In addition, petiole samples were collected from chronically infected grapevines throughout the summer to monitor pathogen populations present in grapevines. Chronically infected grapevines did not test positive for *X. fastidiosa* until July. Similarly, X. fastidiosa-positive-sharpshooters were rare in vineyards until vines began to test positive. Results suggest that secondary (vine-to-vine) spread of *X. fastidiosa* is most likely to occur during July, August, and September in vineyards in the southern San Joaquin Valley.

Understanding the epidemiological role of the vectors of *Xylella fastidiosa* in the Balearic Islands (Spain) by long-term macrocosm and microcosm studies

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The pathogenic bacteria *Xylella fastidiosa* (Proteobacteria: Xanthomonadaceae) was detected in the Balearic Islands in October 2016. In November 2017 EFSA launched a grant for the collection of data and information in the Balearic Islands on biology of vectors and potential vectors of the bacteria *Xylella fastidiosa*. The collection of data includes studies about macrocosm and microcosm. For the long-term study of macrocosm, nine organic farms (three olive, three vineyards and three almond farms) were selected in Majorca. The islands of Minorca, Ibiza and Formentera were sampled twice a year, in summer and autumn. For the microcosm study and in particular for the study of the fecundity of females, 50 cages containing one male and one female of insect vectors and one plant per cage were placed under field conditions. From the macrocosm results, two Aphrophoridae (Hemiptera; Cicadomorpha) species of potential vectors have been detected in the Balearic Islands, *Philaenus spumarius* and *Neophilaenus campestris*. In general, nymphs of Aphrophoridae were more abundant in the cover vegetation of olive crops, followed by vineyard and almond ones. Nymphs of potential vectors were present in the cover vegetation of all crops from early March to the end of May. Adults of Aphrophoridae were more abundant in the cover vegetation from May to June and from October to November; in tree canopies from June to August and in the border crop vegetation from August to October. Adults of *P. spumarius* were more abundant than those of *Neophilaenus* spp. in the different crops. Microcosm results showed that eggs of vectors were found on *Rosmarinus officinalis*, *Lavandula dentata* and *Ocimum basilicum*. We discuss the epidemiological role of the vector species detected in the Balearic Islands, considering their abundance, host preference, seasonality, distribution in the orchards and bionomy.

Keywords: Vectors, epidemiology, bioecology

Modelling the population dynamics of *Philaenus spumarius*: a fully mechanistic approach

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The meadow spittlebug Philaenus spumarius has been identified as spreading the bacterium *Xylella fastidiosa* causing olive quick decline syndrome in Apulia in Southern Italy. Prevention, containment, and local eradication of *Xylella fastidiosa* outbreaks can benefit from vector control to prevent both a pathway for disease spread and the secondary spreading within a crop. Vector monitoring activities and the implementation of mechanical (against vector juveniles) or chemical control (against vector adults) require information on vector population phenology, abundance and activity that are dependent on environmental conditions.

In this work we propose a fully mechanistic model simulating the phenology, age structure and population abundance of *P. spumarius* according to environmental conditions. Local population dynamics is described in terms of physiological responses of the individual life-history strategies (development, survival and reproduction) to local environmental conditions. The non-linear rate functions describing individual physiological responses are estimated based on experiments performed in microcosms at ambient temperature and in climatic chambers at different constant temperatures. The estimated model has been calibrated with data on population dynamics of *P. spumarius* collected in four olive orchards in Northern (Liguria) and Southern (Apulia) Italy from 2016 to 2018.

Model simulations can be used to support vector and disease management. The model can predict the phenology and the abundance of a *P. spumarius* populations at high spatial and temporal resolution. This information can support precision targeting control strategies against vector nymphs and adults. The model can also generate maps of vector phenology and abundance. These maps can represent a source of information for spatial-explicit epidemiological models describing the disease dynamics and supporting *X. fastidiosa* quantitative risk assessment and management. Knowledge on model parameters variability offers the possibility to explore the implication of uncertainties in designing vector and disease monitoring and management strategies.

Keywords: vector control, *Xylella fastidiosa* epidemiology, quantitative risk assessment

Transmission of *Xylella fastidiosa* subsp. *pauca* to olive trees by sharpshooters and spittlebugs common in Brazilian orchards

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The vector-borne bacterium, *Xylella fastidiosa* subsp. *pauca*, has been isolated from olive trees showing olive quick decline syndrome (OQDS) symptoms in different locations of Southeastern Brazil, but the vectors spreading this putative pathogen are unknown in that region. In other crops, this bacterium is transmitted by xylem-sap feeding leafhoppers (sharpshooters) (Hemiptera: Cicadellidae: Cicadellinae) and spittlebugs (Hemiptera: Cercopoidea). In this study, we showed that 11 sharpshooter and three spittlebug species common in olive orchards of the Mantiqueira Mountain Range Region, Southeastern Brazil, are able to transmit *X. fastidiosa* subsp. pauca to olive trees. For the transmission assays, field-collected or laboratory-reared adults of each species were first pretested for natural infectivity by confinement with sleeve cages on healthy seedlings of *Catharanthus roseus* during an inoculation access period (IAP) of 12 h (10-20 insects/plant). After the pretest, the insects were confined with sleeve cages on potted olive trees (cv. Grappolo) infected with *X. fastidiosa* subsp. pauca (sequence type 16) for an acquisition access period of 24 h, followed by a 24-h IAP on potted healthy olive trees cv. Koroneiki (3-10 insects/plant, depending on the sharpshooter/species) in a protected screenhouse. About 3-5 healthy *C. roseus* (pretest) and olive (test) plants were not exposed to insects (negative control). All pretest and test plants were assayed by real time PCR at 12 months after inoculation. Estimated transmission rates by single insects (based on 2-3 trials) ranged from 0.4-12.9%. The following vector species (and transmission rates, when applicable) were identified: the spittlebug *Notozulia entreriana* (9.2%), and the sharpshooters *Erythrogonia phoenicia* (1.6%), *Erythrogonia* sp. 1 (4.8%), *Macugonalia cavifrons* (1.8%), *Macugonalia leucomelas* (1.7%), *Oragua* sp. 1 (0.4%), *Plesiommata* sp. 1, *Scopogonalia paula* (12.9%), *Sibovia sagata* (0.7%) and *Sonesimia grossa* (0.5%). None of them transmitted the bacterium in the pretest assay.

Keywords*:* *Olea europea*, Xylem-limited bacterium, vector range

DNA-barcoding and assessment of the genetic diversity of the *Xylella fastidiosa* vectors in the Balearic Islands

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Since the first detection of *Xylella fastidiosa* (XF) in Majorca (Spain) five years ago, this phytopathogenic bacterium has been found in the rest of the Balearic Islands except Formentera. In the Balearic archipelago, the expansion of the XF infection occurs through the feeding activity of the spittlebugs *Philaenus spumarius* and *Neophilaenus campestris* (Aphrophoridae). To date, these are the only confirmed XF vectors in the Balearic Islands. In 2017 we started a project granted by EFSA aiming at, among other objectives, DNA barcoding putative vectors in the Balearics through the analysis of the standardized 5’ end of the mitochondrial COI gene of the insects. Moreover, a cytB mtDNA fragment was also sequenced in order to expand the genetic dataset and to conduct phylogenetic inferences. We take advantage of the *P. spumarius* and *N. campestris* DNA sequences available at GenBank to investigate the molecular systematics of the Balearic vectors and the patterns of haplotype divergence among vector populations in Europe.

Microbial assemblages within *Philaenus spumarius* and their possible role on insect´s reproduction

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Microorganisms colonizing internal tissues of insects are known important players in the insect's reproduction, nutrition, and fitness. Thus, a better knowledge and characterization of this microbial community can lead to their exploitation in the management of insect populations. *Philaenus spumarius* has been of interest in the recent years due to its confirmed ability of vectoring the plant pathogen *Xylella fastidiosa*. In this work the microbial community of different organs (head, abdomen, and genitalia) of 41 *P. spumarius* was assessed by culturing methods. Overall, 86 isolates classified in 51 operational taxonomic units (OTUs) (41 bacteria and 10 fungi) were obtained, being found 2 OTUs on average per insect. The most abundant genera were *Curtobacterium* (within bacteria) and *Cladosporium* (within fungi). The abdomen has the highest number of microbial isolates and fungal diversity with about 0.32 OTUs per sample, while genitalia harbored the highest bacterial diversity with about 0.68 OTUs per sample. The bacterial isolates able to growt in artificial medium were selected, and their influence on the insect cycle and reproduction were assessed using an insect model. The observed effects on insects were variable depending on the bacterial isolates. Some bacteria demonstrated ability to increase insect population while other bacterial isolates displayed a negative effect by decreasing insect reproduction or by causing insect premature death. Although preliminary, these results are very promising for the management of *P. spumarius* population and, consequently, for the transmission of *X. fastidiosa*.

DAY 5 – Friday 30 APRIL 2021 – 09:00-18.15 CEST

SESSION 3: ***From epidemiological modelling to surveillance and early detection***	
Chairs: Maria Bergsma-Vlami (Netherlands Food and Consumer Product Safety Authority (NVWA) (NL) and Stephen Parnell (University of Salford (UK)	
09.00-09.15	Progress and achievements on the early detection of *Xylella fastidiosa* infection and symptom development with hyperspectral and thermal remote sensing imagery ***Pablo J. Zarco-Tejada**, University of Melbourne (AU)*
09.15-09.30	The EU Reference Laboratory for pests of plants on bacteria and a proficiency test for molecular detection of *Xylella fastidiosa* ***Tanja Dreo**, National Institute of Biology (SI)*
09.30-09.35	Inferring the potential spread of *Xylella fastidiosa* in Great Britain ***Flavia Occhibove** (Young researchers' initiative), UK Centre for Ecology & Hydrology (UK)*
09.40-09.55	Q&A
09.55-10.00	Short break
10.00-10.15	Assessing genome-wide diversity in *Xylella fastidiosa* through target enrichment via hybridization-based capture from natural host plant and insect samples ***María del Pilar Velasco-Amo**, Institute for Sustainable Agriculture, Spanish National Research Council (IAS-CSIC) (ES)*
10.15-10.30	Estimating the rate of spread of Olive Quick Decline Syndrome, caused by *Xylella fastidiosa* subsp. *pauca* in Puglia ***Lia Hemerik**, Wageningen University (NL)*
10.30-10.35	Risk of establishment of Pierce's disease in main wine-producer regions worldwide ***Alex Giménez-Romero** (Young researchers' initiative), Instituto de Física Interdisciplinar y Sistemas Complejos IFISC (CSIC-UIB), Campus UIB (ES)*
10.35-10.50	Q&A
10.50-11.10	**Coffee break**
11.10-11.25	Guidelines for risk-based survey design for *Xylella fastidiosa* ***Sybren Vos**, Animal and Plant Health Unit, European Food Safety Authority*
11.25-11.40	The role of trust in the UK live plant trade: impacts on biosecurity behaviours and the management *of Xylella fastidiosa* risk ***Chris Pollard**, Forest Research (UK)*
11.40-11.45	Media and scientific literature monitoring of *Xylella fastidiosa* using the MEDISYS platform ***Caterina Campese** (Young researchers' initiative), Animal and Plant Health Unit, European Food Safety Authority*
11.45-12.00	Q&A
12.00-12.15	**Announcement of Winner of social media poster competition** announced by Sara Tramontini (*Animal and Plant Health Unit, European Food Safety Authority)*
12.15-14.00	**Lunch break**

Progress and achievements on the early detection of *Xylella fastidiosa* infection and symptom development with hyperspectral and thermal remote sensing imagery

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Remote sensing efforts made as part of European initiatives via POnTE, XF-ACTORS and the JRC, as well as through regional programs, have focused, among others, on the development of algorithms for the early detection of *Xylella fastidiosa* (Xf)-induced symptoms. Airborne campaigns carried out between 2016 and 2019 collected high-resolution hyperspectral and thermal images from infected areas in the Apulia region (Italy), in the province of Alicante and on the island of Mallorca (Spain). The remote sensing imagery collections were performed alongside field surveys and laboratory analyses to assess the presence of Xf, and the severity and incidence of disease in olive and almond trees. Radiative transfer models and machine learning algorithms were used to quantify spectral plant traits for each individual infected tree, assessing their importance as pre visual indicators of Xf-induced stress. These studies conducted across species have demonstrated that specific spectral plant traits successfully revealed Xf induced symptoms at early stages, i.e., before visual symptoms appear. The results show that spectral plant traits contribute differently to symptom detection across host species (olive vs. almond), and that abiotic-induced stress affects the performance of the algorithms used for detecting infected trees. Together, the different European initiatives studying the use of remote sensing to support the monitoring of landscapes for *Xylella fastidiosa* detection lead us to conclude that the early detection of Xf-induced symptoms is feasible when high-resolution hyperspectral imagery and physically-based plant trait retrievals are used, obtaining accuracies exceeding 92% (kappa>0.8). These results are essential to enable the implementation of effective control and management of plant diseases using airborne- drone- and satellite-based remote sensing technologies. Moreover, these large-scale hyperspectral and thermal imaging methods greatly contribute to the future operational monitoring of infected areas at large scales, well beyond what is possible from field surveys and laboratory analyses alone.

EU Reference Laboratory for pests of plants on bacteria and a proficiency test for molecular detection of *Xylella fastidiosa*

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In 2019, the EU designated EU reference laboratory for pests of plants on bacteria. Its first activity, a proficiency test (PT) on molecular detection of *Xylella fastidiosa* (Xyf), aimed to assess proficiency of the EU national reference laboratories (NRLs) was organized in 2019 by the National Institute of Biology. 14 samples were prepared by mixing DNA of *Olea europaea, Coffea arabica, Polygala myrtifolia, Spartium junceum, Lavandula angustifolia, Prunus avium and Vitis vinifera* with DNA of 6 strains Xyf subsp. pauca (3), multiplex (2) or fastidiosa (1). The Xyf copy numbers in the test items corresponded to real-time PCR Cq values reported for real-life samples and were about 104 DNA copies/mL, as determined with digital PCR. Altogether, 26 participants reported 364 individual results of which 97% were conforming. False negative results (3%) were reported for subsp. multiplex and fastidiosa in lavender and grapevine, respectively. The subsp. pauca, causing olive quick decline in Italy, was detected with overall conformity of 100%. Comparative analysis of results showed that, when properly optimized and applied, real-time PCR, PCR and LAMP all enabled correct identification of all test items. Yet points of improvement were identified for seven participants. Overall, results of the PT show that cell densities of Xyf, as commonly anticipated in plant material are readily detected by the EU NRLs regardless of different methods, tests, instruments, reagents, dilutions, etc. This demonstrates that knowledge and competence overrule the need for strict standardization.

Inferring the potential spread of *Xylella fastidiosa* in Great Britain

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Xylella fastidiosa (Xf) is a significant threat affecting the agricultural and horticultural industries worldwide. Once restricted to Americas, severe European outbreaks have been discovered, the most infamously known in Puglia (Italy), where the bacterium is still spreading killing millions of olive trees. It is a great concern due to the high levels of plant trade, but in order to prevent and control its emergence in novel locations, it is vital to understand drivers of entry, establishment, and spatiotemporal spread, which are not fully understood. Great Britain is currently *Xylella*-free, but due to the extensive plant trade network, is considered potentially at risk. Although suitability distribution models (SDMs) suggest unfavourable climatic conditions for Xf emergence there, this approach neglects crucial epidemiological dynamics. Hence, to improve Xf pest risk assessment in Britain, we adapted a spatially explicit mechanistic spread model originally designed to describe the Puglian outbreak, incorporating British specific eco-epidemiological factors. We simulated deterministic short-distance and stochastic long-distance dispersal at country-level, accounting for British environmental gradients and taking a "worst-case scenario" approach to define susceptible host population. Infection was randomly seeded and different scenarios were simulated to evaluate different parameter values, including different dispersal processes, as estimation was uncertain due to lack of epidemiological data. Results showed that temperature was a limiting factor to disease transmission, and host distribution significantly affected local prevalence and extent of spread, suggesting that investigation of spatial dynamics is crucial to reveal areas at risk in case of Xf introduction in new locations. Despite the high degree of parameter uncertainty, our results displayed consistent qualitative patterns, highlighting differences with SDMs and other forms of risk assessment. The model is flexible to be updated whether new data become available, and eventually adapted to other areas, with the ultimate aim of providing guidance to develop preparedness measures.

Keywords: epidemiological model, emerging infectious plant disease, pest risk assessment

Assessing genome-wide diversity in *Xylella fastidiosa* through target enrichment via hybridization-based capture from natural host plant and insect samples

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The taxonomic placement of *X. fastidiosa* (Xf) strains in Europe is of applied relevance, as European Commission-mandated management strategies are based on the subspecies present in each outbreak. Currently, Xf-typing at subspecies and Sequence Type (ST) level is based on the use of MultiLocus Sequence Typing (MLST) analysis. However, genetic resolution at ST-level among strains may not permit the identification of closely related but different strains, indicating that whole-genome sequence (WGS) data should probably be used when considering Xf quarantine, eradication, and containment strategies. Unfortunately, when quickness is a need after detection of an outbreak it is not always possible to isolate the Xf strain to obtain its genome sequence, and the data obtained when using total DNA from infected host plants by MLST or WGS analyses may not succeed or contain enough Xf reads to adequately identify the Xf strain intercepted. In this study, we developed a Xf- Targeted Sequence Capture Enrichment (TSCE) in combination with High-Throughput Sequencing (HTS) using an Illumina platform to provide efficient access to enough Xf genome information to identify Xf at ST level further. More than 7,000 baits targeting 140 Xf gene sequences present in the chromosome or plasmids were selected to cover genomic markers of all Xf subspecies and STs described to date. We showed that whereas < 0.25% of Xf reads were detected by direct WGS of host DNA this number increased up to 41-73% after using the TSCE-HTS approach in individual samples or in mixtures of up to four plant samples. We were able to identify all seven loci commonly used for Xf MLST and correctly identify the subspecies and ST of four different Xf strains. After assembly of captured reads we were able to identify up to 284 Xf coding sequences (CDS) of which 48 to58% corresponded to all of the 140 target sequences used to design the baits, indicating that we were able to capture more CDS than expected. The results obtained from a range of insect and plant samples from different host plants including 'mock samples' or naturally-infected samples and with levels of Xf ranging from very high down to close to detection limit, as determined by Harper's qPCR assays, will be discussed. Next steps include the development of a web-based analysis platform to facilitate implementation of this methodology for its use by different laboratories.

This work was funded by Poject E-RTA2017-00004-C06-02 (AEI-INIA Spain and FEDER), and the Spanish Olive Oil Interprofessional.

Estimating the rate of spread of Olive Quick Decline Syndrome, caused by *Xylella fastidiosa* ssp. *pauca*, in Puglia

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Xylella fastidiosa is a plant inhabiting bacterium that has recently invaded Europe. It was first found in southern Italy in 2013, causing a severe disease in olives. The disease has killed olive trees across entire landscapes. Estimates of spread rate of the disease are needed to plan outbreak control, but empirical estimates of the spread rate were not available. Monitoring data on disease occurrence in Puglia are used to estimate the rate of spread of *X. fastidiosa* in Puglia and to identify the shape of the invasion front. The data have been collected in a non-random spatial pattern that varied greatly from year to year. The data are analysed in different ways, and stochastic simulations are used to demonstrate that the estimation methods used in the end for analysing empirical data give high quality estimates. From comparing different shapes for the invasion front a logistic S-shape was most supported by the data. The rate of movement of the invasion front of *X. fastidiosa* in Puglia is estimated as 9.95 km/year (7.48 – 12.49 km/year). This rate can be used to predict future spread of *X. fastidiosa*, plan control operations and manage the risk of future invasions of this bacterium in southern Italy and elsewhere in Europe.

Risk of establishment of Pierce's disease in main wine-producer regions worldwide

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Pierce's disease (PD) of grapevines caused by *Xylella fastidiosa* is currently restricted to North America with a few recent reports in Taiwan and the island of Mallorca in Spain. The underlying biotic and environmental conditions explaining why PD has not spread worldwide remain unexplained. There have been several attempts to predict the climatic conditions suitable for PD using species distribution models. However, few studies have taken into account the distribution of the vectors, an essential factor for accurate risk assessment. Here we first modelled the climatic effects that determine both the development and survival of Xf within the plant. This includes the effect of climatic oscillations in the initial population dynamics of the infected plants under different basic reproductive number scenarios. Secondly, we accounted for the distribution of the main known vector *Philaenus spumarius* in Europe. We also made predictions in other regions worldwide assuming that the vectors responsible for the disease spreading are abundant enough. The simulation output predicts accurately PD known invaded ranges in the US, Mallorca and Taiwan, and explain the past epidemic patterns in North America. When combined with the distribution of the main vector *P. spumarius*, the risk for chronic PD in Europe is downscaled to unconnected areas of the Mediterranean coast and islands, with much lower risks in the south Atlantic coast.

EFSA’s guidelines for risk-based survey design for *Xylella fastidiosa*

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At the request of the European Commission, EFSA prepared specific guidelines for assisting the surveyor through the design of statistically sound and risk-based surveys for *Xylella fastidiosa* based on the pest’s key biological information. A methodological framework was developed for designing detection surveys to substantiate pest freedom, and delimiting surveys to determine the boundaries of an infested zone. The first step for designing surveys is to define three key parameters: a) the host plant population structure and size; b) the methods used for the detection and identification of the bacterium and their sensitivity, and; c) the aims of the survey in terms of confidence level and design prevalence. In this step, the main challenges posed by the epidemiology of the bacterium, are in particular, that infected plants can be asymptomatic for a long and variable period, that symptoms can be cryptic and resemble drought stress, that the host plant and bacterium association is related to the intraspecific diversity of the bacterium. The second step of the survey design consists of calculating the numbers of inspections, samples and tests to be performed for characterising the entire target population (survey effort). The third step of the survey design is determining where to allocate the survey efforts. The robustness of the surveys conclusions depends strongly on the survey preparation. The proposed approach allows surveys to be tailored to different epidemiological situations, taking into account host plants, the intraspecific diversity of the pest, the vectors, the environmental suitability and the resources available in each Member States. In addition, the resulting surveys when implemented in different locations and periods can be compared, thus contributing to more harmonised *X. fastidiosa* surveys in the EU Member States.

EFSA Working Group on Pest Surveillance: José Cortiñas Abrahantes, Elena Lazaro, Stephen Parnell, Jan Schans, Martijn Schenk, Gritta Schrader, Antonio Vicent Civera, Gabriele Zancanaro

The role of trust in the UK live plant trade: impacts on biosecurity behaviours and the management of *Xylella fastidiosa* risk

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The UK imports over £1bn worth of ornamental plants every year (Defra, 2020). The accidental movement of infected plants through trade pathways is the most likely way the bacterial pathogen *Xylella fastidiosa* would enter and spread within the UK. A key component to manage this risk is to encourage greater biosecurity in the decision-making and actions of people and businesses in the UK plant trade. As part of the BRIGIT Project, we conducted 44 in-depth interviews with a range of stakeholders on decision-making around sourcing and biosecurity actions. Stakeholder groups included were growers, nurseries, retailers, wholesalers, landscape contractors, and consultants. We focussed on traders of one shrub and one tree species which are known to be both *Xylella* host species and are important to the UK ornamentals trade: lavender (*Lavandula* spp.) and sweet cherry (*Prunus avium*). We found that within the live plant value chain, suppliers and customers had long-standing relationships with each other built on trust. This sometimes led to mutually positive biosecurity behaviours but could also lead to risky behaviours. Using the risk-based view of trust framework (Das and Teng, 2004), we: i) characterise the types of trust present in the UK plant trade; ii) identify ways in which these types of trust mediate both positive and negative biosecurity behaviours; and iii) discuss how potential *Xylella* detection and management actions affect and are affected by trusted relationships in the plant trade network.

Keywords: decision-making; biosecurity behaviours; ornamental trade

Media and scientific literature monitoring of *Xylella fastidiosa* using the MEDISYS platform

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The European Commission DG SANTE requested EFSA in December 2016 to carry out a horizon scanning exercise on emerging plant pests of interest for the European Union territory. To answer this request, EFSA extended the automated monitoring system based on the MEDISYS platform that was set up in collaboration with the JRC in the period 2014-2016.

MEDISYS is part of the Europe Media Monitor (EMM) system, fully automatic public health surveillance platform, able to retrieve articles from the internet, categorise them, identify entities (organisations, persons and locations), extract events, cluster articles, and calculate statistics in order to detect emerging threats. Searches are automatically carried out by the system using keywords corresponding to scientific and common names of the pests and associated diseases. The results are collected in monthly newsletters.

Articles on pests regulated in the EU, listed by EPPO and more than 500 newly discovered and emerging plant pests are searched by the platform in more than 12,000 media and scientific literature sources from around 200 countries.

Xylella fastidiosa is the pest most covered by media. Time series of articles are reported to illustrate the media relevance of certain plant health issues related to this pest. The situations in Italy and Spain have been largely covered by media that also reported news about the outbreaks in France, Portugal and the first findings of the pest in Israel. Many additional information about vectors, subspecies and hosts have also been reported by media.

Xylella fastidiosa is also one of the most recurrent pests subject of scientific articles on control measures, surveillance, detection methods and pest epidemiology. Some articles, providing information on new findings, new vectors and new host plants, may be of interest in the context of the regulation of this quarantine pest.

Keywords: horizon scanning, emerging risk, risk preparedness

SESSION 4: ***Towards sustainable management strategies***	
Chairs: Alessandra Alves de Souza (Centro de Citricultura "Sylvio Moreira", Instituto Agronomico (IAC) (BR) and Antonio Vicent (Instituto Valenciano de Investigaciones Agrarias (ES)	
14.00-14.15	Searching for olives displaying resistance traits to *Xylella fastidiosa* subsp. *pauca* ST53: experimental evidence and challenges ***Donato Boscia****, Institute for Sustainable Plant Protection (IPSP), National Research Council of Italy (IT)*
14.15-14.30	Evaluation of alternative compounds against *Xylella fastidiosa* subsp. *pauca* in olive groves of the Salento peninsula of Apulia ***Giuseppe Lima****, Department of Agricultural, Environmental and Food Sciences, University of Molise (IT)*
14.30-14.40	Q&A
14.40-14.45	Short break
14.45-15.00	BIOVEXO, a new BBI-JU-H2020 project on biocontrol of *Xylella* and its vector in olive trees for integrated pest management ***Stéphane Compant****, AIT Austrian Institute of Technology, Center for Health and Bioresources, Bioresources Unit (AT)*
15.00-15.05	Culture and metagenomic approaches for the identification of olive xylem microbial communities as a biological control tool to cope against *Xylella fastidiosa* infection ***Manuel Anguita-Maeso*** *(Young researchers' initiative), Institute for Sustainable Agriculture, Spanish National Research Council (ES)*
15.05-15.10	Screening of a novel biological control agent targeting the phytopathogen *Xylella fastidiosa* ***Marwa Mourou*** *(Young researchers' initiative), Dipartimento di Scienze Agrarie e Forestali (DAFNE), Università degli Studi della Tuscia (IT)*
15.10-15.15	Peptides of synthetic and microbial origin with antimicrobial and antibiofilm activity against *Xylella fastidiosa* ***Luis Alejandro Moll*** *(Young researchers' initiative), Institute of Food and Agricultural Technology-CIDSAV-XaRTA, University of Girona (ES)*
15.15-15.35	Q&A
15.35-16.00	**Coffee break**

Searching for olives displaying resistance traits to *Xylella fastidiosa* subsp. *pauca* ST53: experimental evidence and challenges

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Since 2015, a large-scale olive screening program for identifying sources of resistance to *Xylella fastidiosa* subsp. pauca ST53 (Xfp) was started under natural (field) and controlled conditions (greenhouse, GH) in Apulia (southern Italy). Approx. 100 genotypes including olive cultivars (from 15 different olive-growing countries) and breeding selections were planted in experimental plots located in the demarcated infected area, exposed to natural infections. The panel of tested plant material included also O. europaea subsp. cerasiformis, subsp. guanchica and var. sylvestris. Most of the experimental materials consisted of self-rooted plants propagated from the sources maintained at the World Olive Germplasm Bank and Breeding Program of IFAPA Córdoba (Spain). Sixty genotypes of the plant material under study were also evaluated in GH upon mechanical inoculations.

Field trees were tested once a year and inspected for symptoms twice a year. Potted plants in the GH were monitored periodically to assess symptoms and host bacterial colonization.

Under field conditions, symptoms usually began on the susceptible cultivars during the third year. Colonization was detected since the first year (incidence of 4-20%) and then progressed rapidly reaching values higher than 60% for most of the selections. For few selections, including Leccino, infections were in the range of 20%-40%. After 5 years, shoot dieback/desiccations were evident on trees belonging to all genotypes under testing, with scores ranging from 1 to 2.5 (on a scale 0-5), except only a few showing scores <1.

In the GH tests, within the 3 years of observations, symptoms of desiccations could be observed on the majority of the systemically-infected plants, with symptoms appearing at different times post-inoculation. Infection rates for the majority of the selections ranged from 50% to 89%.

Overall, even if in some genotypes lower incidence of infections and longer asymptomatic periods were recorded, so far none displayed clear phenotypic traits of resistance (low prevalence, symptoms and bacterial population) similar to that observed in the resistant control (Leccino). The data collected from this large screening program showed that the occurrence of genetic traits conferring resistance to Xfp may be very limited in the olive germplasm, emphasizing the need to put in place all measures to restrain and limit its further spread in olive growing areas.

Key words: olive, germplasm, resistance

Evaluation of alternative compounds against *Xylella fastidiosa* pauca in olive groves of the Salento peninsula of Apulia

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Xylella fastidiosa pauca (Xfp), strain ST53, is the causal agent of the Quick Decline Syndrome, a new serious bacterial disease of the olive trees found in olive groves in southern Italy since 2013. The phytopathogenic bacteria is efficiently spread by vector insects and then millions of olive trees in the Salento peninsula are infected with the pathogen. Disease control and limitation of its vectors are very difficult as traditional antibacterial products (e.g., copper contact products) are ineffective and vectors are polyphagous and ubiquitous. The use of resistant germplasm and the adoption of quarantine measures are currently the most effective measures. However, to protect Xfp-susceptible olive cultivars of the Salento peninsula (Cellina di Nardò and Ogliarola Salentina), including also a high number of monumental trees with high environmental and cultural value, an integrated approach, based on adoption of preventive agronomic measures together with new effective and environmentally-safe products, are needed. To this aim in different olive groves, we are evaluating canopy and soil treatments with novel formulates (fertilizer, biostimulant and/or systemic antibacterial products). In a three-year investigation in a olive nursery and groves, a mixture of seaweed and plant polyphenols (SWP) alone or in combination with zinc and copper systemic products (Zn-Cu), applied 4 times per year, showed significantly lower incidences of Xfp symptoms compared to untreated controls. Furthermore, the SWP formulate, particularly when applied to the soil, increased plant biomass and leaf content of macro and microelements (i.e., P, K, Mg, Zn, Bo, Mn and Mo). Further experiments will evaluate the effect of these products on Xfp host-colonization and their possible mechanisms of action.

BIOVEXO, a new BBI-JU-H2020 project on biocontrol of *Xylella* and its vector in olive trees for integrated pest management

Compant S

AIT Austrian Institute of Technology, Center for Health and Bioresources, Bioresources Unit, Tulln, Austria and BIOVEXO Consortium*

Xylella fastidiosa is increasingly causing diseases on olive trees and various other crops in the Mediterranean region. It wiped out a number of olive groves in Italy and Spain in only a few years. Unfortunately, the climate of the southern European Union is ideal for *Xylella*. Due to its rapid transmission across cultivation areas, *Xylella* is projected to cause yield losses of 35% - 70% in olive harvests and 13% in almond harvests. Currently, there are no pesticides available on the market proven to be effective against *Xylella*, which is spread by xylem-feeding insects – notably the spittlebug *Philaenus spumarius*– common in the Mediterranean climate. Farmers are often forced to destroy infected plants or use chemical insecticides, damaging incomes as well as organic production. In the BBI JU-H2020 project BIOVEXO (1), 11 partners from 5 different countries and belonging to industries, SMEs, RTOs and universities aim to develop environmentally sustainable and economically viable plant protection solutions that can be deployed as a method of integrated pest management to control *Xylella* and its spittlebug vector. At least, the two best performing solutions will be brought forward, closer to the market, at the end of the project (Technical Readiness Level 7-8). Integrated pest management measures will be applied to the existing, and the newly planted orchards during the large-scale pilot in Apulia (Italy) and Mallorca (Spain) — the two most dominant *Xylella* outbreak regions in Europe. In the conference, we will present the overview of the project as well as the objectives and the research plan of BIOVEXO.

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For more information about the BIOVEXO project: www.biovexo.eu.

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Culture and metagenomic approaches for the identification of olive xylem microbial communities as a biological control tool to cope against *Xylella fastidiosa* infection

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The xylem-inhabiting plant pathogenic bacterium *Xylella fastidiosa* (Xf) represents one of the major Phytopathological threats to olive crop worldwide, due to its devastating effects on agricultural yields losses and high tree mortality that causes profound socioeconomic and environmental impacts. Endophytes play an essential role on plant growth and its physiological status, but they can also act as an innate natural defense to cope against infection by xylem-inhabiting pathogenic organisms. Today, vast majority of microorganisms residing in olive xylem are unknown; therefore this work pursues the characterization of the olive microbiome through culture-dependent and independent (NGS) techniques as a tool for identifying potential biological control agents for this pathogen. Hence, four cultivated olive genotypes (Arbequina, Arbosana, Koroneiki and Grappolo) located in Sao Paulo state (Brazil) showing visual Xf symptoms or asymptomatic-non-infected were selected. Xf infection was verified by qPCR. For the culture-dependent approach, chips extracts of xylem tissue from branches and roots were plated in two solid media (R2A and R2A supplemented with plant extract). For culture independent approach, total DNA extracted from xylem tissue was analyzed by metagenomic analysis of 16S and ITS region to characterize the xylem-inhabiting bacterial and fungal communities. Preliminary culture results indicated differences in the frequency of microbial communities depending on the olive genotype and the type of plant material analyzed, as well as, the presence or absence of Xf symptoms on the sampled trees that correlated with Xf infection. These results will help to expand our knowledge on the olive xylem microbiome community composition and understand its driving factors when Xf infection occurs and more importantly to identify xylem-inhabiting microorganisms with potential to combat this harmful bacterium.

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Screening of a novel biological control agent targeting the phytopathogen *Xylella fastidiosa*

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In autumn 2013, a disease outbreak affecting olive trees occurred in Gallipoli, a restricted area in the southern of Italy. It was the occurrence of the Olive Quick Decline Syndrome (OQDS) caused by a devastating xylem bacterium, the so called *Xylella fastidiosa* subsp. pauca De Donno strain. In spite of the pertinent research effort and the several published experiments devoted to control the disease, so far there are no control measure available to eliminate the bacteria from a diseased host plant. Therefore, the aim of this study was the screening of antagonistic activity of epiphytic bacteria isolated from the phyllosphere of 8 different host plant species of Xf *(Olea europaea, Polygala myrtifolia,, Rosmarinus officinalis, Nerium oleander, Laurus nobilis, Myrtus communis, Prunus Dulcis and Prunus avium*)
In vitro dual culture tests showed that 12 out of 89 isolates inhibited Xf growth with an appearance of clear zones between 4.0 and 24.0 mm. Biochemical tests and 16S rRNA gene sequencing revealed different bacterial species belonging to *Pantoea agglomerans* (1), *Microbacterium oxydans* (1), *Microbacterium* spp. (2), *Microbacterium oleivorans* (1), *Microbacterium phyllosphaerae* (1), *Stenotrophomonas rhizophila* (1), *Curtobacterium flaccumfaciens* (1), *Sphingomonas molluscorum* (1), *Delftia acidovorans* (2) and *Pseudomonas graminis* (1). Based on results obtained in this study, among the 12 isolates able to reduce the growth of *X. fastidiosa*, *Pantoea agglomerans* being the most efficient. Taking into consideration that *Pantoea agglomerans* and *Delftia acidovorans* have potential ability to cause human infections, this aspect will be carefully monitored and, as clarified, the antagonistic efficacy of all isolates will be tested in vivo and under natural field conditions.

Keywords: Epiphytic bacteria, De Donno strain, antagonistic activity

Peptides of synthetic and microbial origin with antimicrobial and antibiofilm activity against *Xylella fastidiosa*

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Xylella fastidiosa is one of the most harmful bacterial plant pathogens worldwide, causing a variety of diseases in different host plants, with a huge economic impact on agriculture and the environment. Nowadays, the measures adopted in Europe are eradication of the infected plants, combined with the application of insecticides to control the vector population, and the use of pathogen-free plant material. These methods have been only partially successful and, therefore, there is a need for new treatments for disease control in infected plants.

Antimicrobial peptides of synthetic or microbial origin could be considered promising candidates. These peptides exhibit high activity against a wide range of plant pathogens, low toxicity, high biodegradability and a mode of action that hinders the development of bacterial resistance. Moreover, they also possess antibiofilm properties. Since *X. fastidiosa* is a xylem-limited pathogen that can occlude sap flow in the xylem vessels by biofilm development, peptides displaying both activities are interesting candidates to control the disease.

In this work, peptides with antibacterial and antibiofilm properties have been selected from the literature and synthesized. Also, peptides of bacterial origin have been obtained from cultures such as *Bacillus* spp., *Pantoeas* spp. and *Pseudomonas* spp. of selected strains from our collection. All these sequences have been tested for their antibacterial and antibiofilm activities against X. fastidiosa. Antibacterial activity was determined using a contact test combined with a viability q-PCR. Antibiofilm activity was tested by biofilm quantification using violet crystal staining. Haemolytic activity of the peptides was evaluated by exposing them to horse erythrocytes and phytotoxicity was assessed by infiltrating them into tobacco leaves.

Peptides with a good biological activity profile in terms of high antibacterial and/or antibiofilm activity and moderate to low toxicity have been identified.

SESSION 5: ***What priorities for research on*** **Xylella fastidiosa*****?*** ***Science support to stakeholders needs***	
Chairs: **Baldissera Giovani** (Euphresco) and **Giuseppe Stancanelli** (Animal and Plant Health Unit, European Food Safety Authority)	
16.00-16.10	*Xylella fastidiosa* knowledge gaps and research priorities: contributions from the European *X. fastidiosa* conferences ***Giuseppe Stancanelli****, Animal and Plant Health Unit, European Food Safety Authority*
16.10-16.20	New research priorities on *Xylella fastidiosa* from EU farmers perspective ***Anna Rufolo****, COPA-COGECA*
16.20-16.30	New research priorities on *Xylella fastidiosa* from EU nurserymen perspective ***Josep M. Pagès****, European Nurserystock Association (ENA)*
16.30-16.40	What research needs in the outbreak areas? A nursery perspective for olive and fruit trees propagation ***Luigi Catalano****, CIVI-Italia (IT)*
16.40-16.45	Short break
16.45-16.55	What research needs in the outbreak areas? A risk manager perspective ***Vicente Dalmau Sorli****, Generalitat Valenciana (ES)*
16.55-17.05	The new legal framework of the European Union on *Xylella fastidiosa* and future research priorities ***Rosalinda Scalia****, European Commission DG SANTE*
17.05-17.40	Discussion on stakeholders research needs with the Chairs of the 3rd European conference on *Xylella fastidiosa*
17.40-17.45	Short break
17.45-18.00	Horizon Europe and Plant Health – opportunities for *Xylella fastidiosa* R&I ***Gisela Quaglia****, European Commission DG AGRI*
18.00-18.15	Conclusions and closure of the 3rd European conference on *Xylella fastidiosa* ***Maria Saponari****, Institute for Sustainable Plant Protection (IPSP), National Research Council of Italy (IT) - Coordinator of the H2020 XF-ACTORS project* ***Claude Bragard****, Université catholique de Louvain - Chair of EFSA Scientific Panel on Plant Health*

Abstracts of this session will be added to the final version of the Book of Abstracts.

E-poster session

Blue-shaded posters are contributions from the H2020 XF-ACTORS project.

POSTER SESSION:
Pathogen biology, ecology and genetics

Title	Presenter	DOI
Pathogenicity of *Xylella fastidiosa* subsp. multiplex isolates from Alicante outbreak (mainland Spain) on different hosts	**Domingo-Calap ML**, IVIA, Valencia (ES)	**https://doi.org/10.5281/zenodo.4678347**
Characterization of anti-*Xylella* endolysins from genomic data. A preliminary insight into the identification of novel antimicrobial molecules	**Rosselli R**, University of Alicante, Alicante (ES)	**https://doi.org/10.5281/zenodo.4674618**
Natural competence and homologous recombination among *Xylella fastidiosa* strains	**Liu R**, Auburn University, Auburn (US)	**https://doi.org/10.5281/zenodo.4678393**
Application of phage display: development of tools to fight against *Xylella fastidiosa*	**Favelin N**, Génie Enzymatique et Cellulaire (CNRS UMR 7025), Université de Technologie de Compiègne (FR)	**10.5281/zenodo.4678824**
Genetic diversity of *Xylella fastidiosa* subsp. *fastidiosa* after invasion to a new region	**Tsai CW**, National Taiwan University, Taipei (TW)	**https://doi.org/10.5281/zenodo.4678826**
Csp1, a cold-shock protein homolog in *Xylella fastidiosa* is involved in stress response and biofilm formation*	**Wei W**, USDA Parlier, CA (US)	**https://doi.org/10.5281/zenodo.4678828**
Xylella fastidiosa in the Balearic Islands: a genetic diversity hotspot in Europe	**Moralejo E,** Empresa de Transformación Agraria (Tragsa), Delegación de Baleares, Palma de Mallorca (ES)	**https://doi.org/10.5281/zenodo.4679324**
Phenotypic characterization of Spanish strains of *Xylella fastidiosa* subsp. fastidiosa ST1	**Velasco-Amo MP,** Institute for Sustainable Agriculture, Spanish National Research Council (IAS-CSIC), Córdoba (ES)	**https://doi.org/10.5281/zenodo.4679328**
Detection of recombination events in *Xylella fastidiosa* genomes of different Spanish strains	**Arias-Giraldo LF**, Institute for Sustainable Agriculture, Spanish National Research Council (IAS-CSIC) (ES)	**https://doi.org/10.5281/zenodo.4679344**
MqsR-dependent regulon of *Xylella fastidiosa* modulates stress tolerance and persister cell formation	**Carvalho IGB**, Centro de Citricultura Sylvio Moreira Agronomic Institute (IAC) (BR)	**https://doi.org/10.5281/zenodo.4679346**

A model and image based investigation of *X. fastidiosa* within host dynamics*	**Walker N,** Bioengineering Sciences Research Group, Faculty of Engineering and Physical Sciences, University of Southampton, Southampton (UK)	**https://doi.org/10.5281/zenodo.4679488**
The challenge of searching for *Xylella fastidiosa* genetic diversity in its natural habitats	**Moralejo E**, Empresa de Transformación Agraria (Tragsa), Delegación de Baleares, Palma de Mallorca (ES)	**https://doi.org/10.5281/zenodo.4679495**
Population dynamics of *Xylella fastidiosa* subsp. *pauca* indicate that coffee strains are the founding populations for the olive-*Xylella* infections in Brazil	**Coletta-Filho HD**, Centro APTA Citros Sylvio Moreira, Instituto Agronômico, Cordeirópolis (BR)	**https://doi.org/10.5281/zenodo.4671436**

**abstracts selected through the Young researchers' initiative are reported in the oral presentations section only.*

Pathogenicity of *Xylella fastidiosa* subsp. *multiplex* isolates from Alicante outbreak (mainland Spain) on different hosts

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In the outbreak of *Xylella fastidiosa* in the province of Alicante (mainland Spain), all isolates characterized so far belong to subsp. multiplex and ST6, and the main host is *Prunus dulcis*. Two different strains (IVIA 5901 and ESVL), isolated from almond trees in two municipalities of the Demarcated Area, were used to carry out pathogenicity tests on different plant species. The Italian strain De Donno (subsp. *pauca*, ST53) and the Spanish strain IVIA 5770 (subsp. *fastidiosa*, ST1) were also included. Five cultivars of *Prunus dulcis*, eight of *Olea europaea*, four of *Vitis vinifera*, *Citrus* x *limon*, *Citrus* x *sinensis*, *Citrus reticulata*, *Diospyros kaki*, *Eriobotrya japonica*, and the forest species *Quercus suber* and *Q. ilex* were challenged. A total of 35 plants for each species or cultivar were inoculated. At 1, 3, 6, 12 and 18 months post-inoculation (mpi) plants were sampled and analyzed by real-time PCR, to assess bacterial movement over time. The results show that, in general, no clear pattern of movement was found in the different plant species. Interestingly, in olive and grape, regardless the cultivar, the subsp. *multiplex* ST6 Alicante isolates were hardly detected from 12 mpi onwards, i.e. the systemic movement was very slow. In fact, they were only detected in less than 4% of the infected plants and with very late Cq values. In citrus plants, detection of these isolates failed since 3 mpi, as occurred in kaki and loquat trees. As for *Quercus* species, the detection rate was higher in *Q. suber* than in *Q. ilex*. Only in almond and cork oak trees, leaf scorch symptoms could be correlated with the presence of *X. fastidiosa* subsp. *multiplex*. Moreover, in these cases the pathogen was re-isolated. The timing and the level of detection for all the strains throughout the trial obtained so far are presented.

Keywords: *multiplex*, pathogenicity, hosts

Characterization of anti-*Xylella* endolysins from genomic data. A preliminary insight into the identification of novel antimicrobial molecules

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Xylella fastidiosa is an emerging problem among infective agents threatening crops of commercial interest in the Mediterranean region, most notably Eastern Spain and Southern Italy. Infections determined by *X. fastidiosa* subspecies *multiplex* and *X. fastidiosa* subspecies *pauca*, affect respectively almond and olive trees, and represent a major concern in these two countries. Ecological and economic damages that *Xylella* spp. can cause, therefore, recommend measures aimed at preventing further spreading across different plants and areas of agricultural interest.

Among molecules with intrinsic antimicrobial activity, phage-encoded endolysins constitute a natural tool which may be used in the biological warfare against pathogenic microbes. Lytic and lysogenic phages express endolysins at the late stage of the infection in order to degrade the host cell wall and favor the dispersal of viral particles in the environment. A number of phages capable of infecting *Xylella* spp. have been already sequenced, and lysogenic phages can be identified from available *Xylella* spp. pure culture genomes. Thus far, we have identified hundreds putative endolysins from genomic data with the aim of exploring their efficiency as antimicrobial agents against *X. fastidiosa*. We found 159 candidate anti-*Xylella* endolysins belonging to four mayor clades with possible differential specificity against *Xylella fastidiosa* subspecies. Among these proteins, we selected a subset of interest for biotechnology application based on distinctive features of their sequences and predicted protein structures.

Clearly, new genomic data from further isolates of *Xylella fastidiosa* and relative phages will extend the information regarding spreading and diversity of this plant pathogen. At the same time, this information will also increase the available database of endolysins for the identification and testing of novel candidate anti-microbial molecules.

Keywords: Endolysins, genomics, phages

Natural Competence and Homologous Recombination among *Xylella fastidiosa* strains

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Horizontal gene transfer (HGT) contributes to genetic diversity and can influence adaptation of plant pathogens to the environment. Natural competence (NC) is one of the mechanisms of HGT that relies on uptake and recombination of extracellular DNA. To date, NC among *X. fastidiosa* has been described only for a few strains. The presence of NC in diverse *X. fastidiosa* strains and how it correlates with recombination detected on whole-genome sequence comparisons remains unknown. Here we characterized NC in 71 *X. fastidiosa* strains from all subspecies - mainly subsp. *fastidiosa* (41 strains) and *multiplex* (26 strains) - by measuring plasmid pKLN61 recombination efficiency. Recombination rates above the detection limit were found in 45 out of 71 tested strains. Results show that the majority of tested subsp. *fastidiosa* strains (96%) are naturally competent, while only 23% of tested subsp. *multiplex* strains show NC. Interestingly, none of the 6 European strains we tested show NC. Additionally, we analyzed homologous recombination in the core genome of 154 *X. fastidiosa* strains. Contrasted to the NC experimental results, many subsp. *multiplex* strains from Southeastern US that are not recombinant when tested in vitro, show comparatively high frequencies of recombination events in their genome. These results demonstrate variability of NC among *X. fastidiosa* populations.

Genetic diversity of *Xylella fastidiosa* subsp. *fastidiosa* after invasion to a new region

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The economically important plant pathogen *Xylella fastidiosa* has been reported in multiple regions of the globe during the last two decades, threatening a growing list of crops and industries. *Xylella fastidiosa* subspecies *fastidiosa* causes disease in grapevines (Pierce's disease of grapevines, PD), a current problem in North America, Europe, and Asia. In this research, we studied PD-causing subsp. *fastidiosa* populations and compared the genome sequences of 33 isolates found in central Taiwan with a larger databased of 176 isolates from the US and two from Spain, the path of introduction into Taiwan was also investigated. Phylogenetic relationships, haplotype network, and genetic diversity analyses confirm that *X. fastidiosa* subsp. *fastidiosa* was recently introduced into Taiwan from the Southeast US (Georgia based on available data). Recent core genome recombination events were detected among introduced *X. fastidiosa* subsp. *fastidiosa* isolates in Taiwan. The events originated from an unknown donor not included in the current sampling, suggesting that higher sequence diversity exists in the region. Notably, no recombination events were detected between subsp. *fastidiosa* and *Xylella taiwanensis*. In summary, the results demonstrate that PD-causing *X. fastidiosa* population in Taiwan originated from the Southeast region of the US, and recombination occurs among isolates in Taiwan after invasion. This study will improve our understanding of the genetic diversity of PD-causing *X. fastidiosa* after invasion to a new region.

Keywords: genomic diversity, recombination, Pierce's disease

Xylella fastidiosa in the Balearic Islands: a genetic diversity hotspot in Europe

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The Balearic Islands hold one of the major genetic diversity of the pathogen *Xylella fastidiosa* in Europe. To date four genetic lineages, ST1, ST7, ST 80 and ST81, belonging to the three formally known subspecies, *fastidiosa*, *multiplex* and *pauca* have been detected in the islands. Recently, we have unravelled the origin of two of these introductions in the island of Mallorca, the ST1 of subsp. *fastidiosa* and the ST81 of subsp. *multiplex*. They were associated with a long-overlooked outbreak of almond leaf scorch disease traced back to 1993 (Moralejo et al. 2020). Less is known about the origin of the ST80 (subsp. *pauca*) in Ibiza, although there is circumstantial evidence that would indicate it was introduced through the ornamental plant trade at least one decade ago. The ST80 produces a lethal dieback of olive trees; however, they seem less virulent towards olive trees than its counterpart ST53 in Apulia, Italy. Nonetheless, it poses a considerable threat to mainland olive oil production. The other main threat is the ST1 responsible of Pierce's disease, which is still confined in the island of Mallorca (Moralejo et al. 2019). We used a large database of more than 13,000 samples analysed since 2016 to highlight general aspects of *Xylella's* biology, including population genetics, epidemiology, landscape impact, dispersal, phylogenetic signal in host range and disease control. Most of the characteristics related to Pierce's disease and almond leaf scorch disease repeat those general patterns observed in California, but others seem to be specific to the Balearic Islands.

Phenotypic characterization of Spanish strains of *Xylella fastidiosa* subsp. *fastidiosa* ST1

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Xylella fastidiosa (Xf) strains from Mallorca belong to subspecies *fastidiosa* Sequence Type (ST) 1. Isolates from Xf subsp. *fastidiosa* cause Pierce's Disease on grapevine and leaf scorch disease on almonds in California. Xf colonization and disease development in host plants have been shown to be related to the size of cell aggregates, bacterial motility, and biofilm formation, which are mediated by Type I and Type IV pili, among other traits. In this study several Xf subsp. *fastidiosa* strains from Spain were phenotypically characterized and compared with strains from the USA. The four Spanish Xf strains used included IVIA5235, IVIA5770, XYL461, R2XF4358 that were isolated from cherry tree, grapevine and *Rhamnus alaternus*. The Californian strains included Temecula 1 (reference strain), WM1-1 and CFBP7970 that were isolated from grapevines; M23, CFBP8073, CFBP7969, CFBP8068, CFBP8082 isolated from almonds, *Coffea canephora*, *Vitis rotundifolia*, *Ulmus* sp., and *Ambrosia artemifolia*, respectively. To study bacterial behavior and phenotypic characteristics several experiments were performed to determine adhesion force to substrate, biofilm formation, movement, cell–cell aggregation, twitching motility and patterns of bacterial growth. Taking together our results showed that Spanish strains do not have twitching motility as M23, CFBP7969, CFBP8068, CFBP8082 strains, whereas the remaining strains have. Standardized area under the growth progress curve showed that IVIA5770 and IVIA5235 have similar growth rate and planktonic growth than M23, but lower biofilm formation as compared to the remaining strains. Future research will be performed to determine the relationship between those phenotypic traits and virulence level on host plants of agronomic interests.

This work has received funding from COST Action CA16107 EuroXanth supported by European Cooperation in Science and Technology727987 XF-ACTORS (H2020-UE) and E-RTA2017-00004-C06-02 from AEI-INIA Spain and the Spanish olive oil Interprofesional.

Detection of recombination events in *Xylella fastidiosa* genomes of different Spanish strains

Arias-Giraldo LF[1], Potnis N[2], De La Fuente L[2], Moralejo[3] E, Marco E[4], Velasco-Amo MP[1], Román-Écija M[1], Imperial J [1], Landa BB[1]

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Xylella fastidiosa (Xf) has the capacity of uptaking extracellular DNA from the environment and incorporate it into its genome by homologous recombination, process known as Natural Competence. The process of genetic acquisition and recombination of extracellular DNA could be related to the acquisition of new traits such as antibiotic resistance and virulence factors, giving rise to the origin of new pathotypes. We took a step forward in comparative genomics analysis of Spanish populations of Xf by determining the complete genome of several isolates from Alicante, Mallorca and Ibiza and established potential recombination events that may have occurred with isolates from other places in the world. For this purpose, we used combined Illumina and Oxford Nanopore sequencing platforms and combined assembly approaches to obtain complete circularized genomes. Our results showed that olive and almond isolates belonging to subspecies *pauca* ST80 from Ibiza (Balearic Islands), the only place where this ST has been detected, showed an unusual level of recombination, being so far the isolates showing the higest number of recombination events among all the Xf isolates sequenced to date. On the order hand, we have confirmed the high degree of homology among genomes from Xf strains from Mallorca and California belonging to subspecies *fastidiosa* ST1 and subspecies *multiplex* ST81 isolated from almond and grapes, and we have also identified specific long-size genomic rearrangements in isolates of Xf subspecies *multiplex* ST6 from Alicante, compared to their homologous strains from the Americas. Research is now underway to determine the source and the functionality of these recombinant genes and their role in pathogenicity.

Acknowledgement: This work has received funding from COST Action CA16107 EuroXanth supported by European Cooperation in Science and Technology727987 XF-ACTORS (H2020-UE) and E-RTA2017-00004-C06-02 from AEI-INIA Spain and the the Spanish olive oil Interprofesional.

MqsR-dependent regulon of *Xylella fastidiosa* modulates stress tolerance and persister cell formation

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Bacterial toxin-antitoxin (TA) systems have been recently characterized for their role on persistence, biofilm formation and pathogenicity. The MqsRA TA system has been shown to modulate these traits in the plant pathogen *Xylella fastidiosa*, a Gram-negative bacterium responsible for economic damage in several crops worldwide. Here we identified the genetic mechanisms regulated by *X. fastidiosa* MqsRA, identifying changes in global gene expression by RNA-Seq in *X. fastidiosa* overexpressing MqsR toxin under control of its native promoter. In addition, cells were treated with copper to assess changes in gene expression in response to this wide used pesticide. The results showed that many genes were differentially expressed in response to MqsR, including those associated to stress tolerance, persister cell formation, transcriptional regulation, cell movement, adhesion and aggregation. Although similarities were observed with the MqsR-dependent regulon of *E. coli*, specific genes of *X. fastidiosa* were differentially expressed, particularly those associated with persister cell formation, proteolysis and transcriptional regulation. Overall, MqsR transcriptionally modulates the formation of persister cells and tolerance during copper stress. These results indicate that MqsRA has a key role in modulate specific genes for *X. fastidiosa* adaptation and survival in environmental stress.

The challenge of searching for *Xylella fastidiosa* genetic diversity in its natural habitats

Moralejo E

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From a mycologist and plant pathologist perspective with little background on prokaryotes, the lifestyle of *Xylella fastidiosa* (Xf) is still fascinating and enigmatic. In recent years, several studies have largely improved our understanding on the genetic diversity and phylogeny while unraveling the center of origin of the three main Xf subspecies. Yet, surprisingly, these works rely exclusively on isolates collected from human-related environments, i.e., crops, ornamental and landscape plants, out of their native geographic range and mostly from non-native hosts. Such scientific gaps hamper our interpretation on the new advances in the biology of Xf and its vectors. We must therefore recognize the paucity of our knowledge on the ecological niche in its native range and the need to survey in candidate ecosystems such as tropical rain forests of Central and South America. The purpose of this work is to generate debate on how to approach the problem of searching for Xf isolates in natural ecosystems. Although at first glance it might seem an easy task, the challenge requires some previous thoughts. To increase our chances of finding the needle in the haystack, we must ask ourselves what role Xf might have in its natural habitat. This needs a reverse path of ecological inference from the collection of scattered information from the lab and observations in the realized niche. In other words, we need to speculate on the evolutionary ecology of Xf in its natural habitat on the base of what we have. There are many questions and few solid answers about the interspecific interactions, host range, geographic size and population genetics, among other issues. These answers must be integral, cohesive and open-minded because our preconceptions can be wrong. Understanding Xf's ecological niche in its natural habitat would aid to evaluate future risks and improve our control strategies.

Population dynamics of *Xylella fastidiosa* subsp. *pauca* indicate that coffee strains are the founding populations for the olive-*Xylella* infections in Brazil

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Xylella fastidiosa subsp. *pauca* (X.f. *pauca*) is a multi-host pathogen responsible for significant disease outbreaks in the agro-ecosystem worldwide. In Brazil, the species causes severe economic losses in coffee and sweet orange productions and was recently identified as causing the olive quick syndrome decline (OQSD), a newly emerging disease. We lack information on the evolutionary potential of this new olive-infecting population, and its relationship with established populations from other hosts. We showed that X.f. *pauca* strains were widely spread through olive orchards and 75% of the diseased plants were infected by a single sequence type (ST16) that was firstly reported infecting coffee orchards. Based on the epidemiological characteristics of X.f. *pauca* and its ancient infections in coffee plants in Brazil, we then hypothesized that coffee populations are the source of inoculum of X.f. *pauca* for olive trees under natural conditions. To test this hypothesis, we analyzed the genetic diversity and population dynamics of all 584 isolates (n=82 from olive, n=170 from coffee, and n=237 from citrus) of X.f. *pauca* using 12 microsatellites (SSR) loci. We observed that the coffee-infecting population had the highest genetic diversity and lowest clonal fraction among the three hosts. Based on a discriminant analysis of principal components and Bayesian clustering analysis, we found evidence for two major genetic groups of isolates. The first group is formed by isolates from olive and coffee and the second and more distant by isolates from citrus. A minimum spanning network analysis provided further evidence for the proximity between olive and coffee populations, maintaining citrus haplotypes further isolated. Coffee and olive crops occur sympatrically in São Paulo and Minas Gerais states, which may favor the exchange of the genetic material between these two hosts. Our results corroborate the importance of monitoring the population structure of multi-host pathogens to elaborate strategies for pathogen control and reduction of pathogen transmission.

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POSTER SESSION:
Host plant-interactions and search for resistant/tolerant germplasm

Title	Presenter	DOI
Broth media cultivation of xylem microbiome from cultivated olive trees	**Anguita-Maeso M**, Institute for Sustainable Agriculture, Spanish National Research Council (IAS-CSIC), Córdoba (ES)	**https://doi.org/10.5281/zenodo.4672520**
Transcriptome profiling of two coffee varieties in response to infection with an endemic *X. fastidiosa* subsp. *fastidiosa* strain from Costa Rica	**Chacón-Díaz C**, Universidad de Costa Rica, San José (CR)	**https://doi.org/10.5281/zenodo.4673078**
Study of the complementation of *xylella fastidiosa* causing CVC with a functional polygalacturonase enzyme and its impact on bacterial physiology	**Costa MRL,** Centro de Citricultura Sylvio Moreira Agronomic Institute (IAC), Cordeirópolis (BR)	**https://doi.org/10.5281/zenodo.4674519**
The new update of the European Food Safety Authority database of *Xylella* spp. host plant species	**Delbianco A,** Animal and Plant Health Unit, European Food Safety Authority, Parma (IT)	**https://doi.org/10.5281/zenodo.4673057**
Attempts for improving protocol to phenotype olive cultivars responses to *Xylella fastidiosa* infections	**Loconsole G**, Institute for Sustainable Plant Protection, CNR, Bari (IT)	**https://doi.org/10.5281/zenodo.4672469**
Genetic characterization of different Vitis species using SSR markers associated with the resistance gene PdR1	**Martínez-Cabero S,** NEIKER- Instituto Vasco de investigación agraria, Arkaute (ES)	**https://doi.org/10.5281/zenodo.4674318**
Search for crop species immune to *Xylella fastidiosa* subsp. pauca, ST53	**Montilon V,** University of Bari Aldo Moro, Department of Soil, Plant and Food Sciences, Bari (IT)	**https://doi.org/10.5281/zenodo.4674457**
Evaluation of xylem vascular occlusions in olive cultivars infected with *xylella fastidiosa*	**Montilon V,** University of Bari Aldo Moro, Department of Soil, Plant and Food Sciences, Bari (IT)	**https://doi.org/10.5281/zenodo.4674328**
Pathogenicity and systemic colonization of Spanish strains of *Xylella fastidiosa* subsp. *multiplex* and *pauca* on olive under controlled conditions	**Román-Écija M**, Institute for Sustainable Agriculture, Spanish National Research Council (IAS-CSIC), Córdoba, (ES)	**https://doi.org/10.5281/zenodo.4672355**
The Arabidopsis immune receptor EFR increases resistance to Xanthomonas and *Xylella* in transgenic sweet orange	**Teixeira-Silva NS,** Sylvio Moreira Citrus Research Center, Agronomic Institute of Campinas, Cordeirópolis (BR)	**https://doi.org/10.5281/zenodo.4674394**

Broth media cultivation of xylem microbiome from cultivated olive trees

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Nowadays there is no culture media described for axenic growth of olive xylem microbiome which can be essential to understand the ecological interactions of xylem-inhabiting pathogens. This work focused on the study of culture media of defined composition to sustain growth of xylem-inhabiting microorganisms. Xylem sap extracted with a Scholander pressure chamber from two olive cultivars were inoculated in SMX, XVM2, XF26, PD3, 3G10R and XDM2 liquid culture media for two weeks at 28°C. The increase in absorbance was measured daily and aliquots were sampled every two days to analyze changes in bacterial composition by Illumina MiSeq metagenomic analysis. Globally, the most abundant genus of culturable bacteria was *Massilia* (21.25%), followed by *Sphingomonas* (20.54%), *Curtobacterium* (9.80%), *Pseudomonas* (7.51%) and *Frigoribacterium* (7.12%), although there were substantial differences in microbial communities over time and among culture media. Thus, SMX and XF26 displayed the highest bacterial OTU richness variation on time (33-9 and 30-6, respectively), followed by XVM2 (29-11), XDM2 (25-9), 3G10R (19-9) and PD3 (9-6). Currently, we have evaluated the possibility to modify the xylem microbiome composition of olive seedlings by transplanting a microbiome artificially cultured in liquid media by an endotherapy treatment and monitored the stability of this modification over time. Results indicated that consortia of xylem microorganisms can be cultivated in vitro using predefined culture media, and that those can artificially introduced in xylem vessels of seedling plants modifying their native xylem microbiome. This technique, when optimized, can settle the basis to inoculate ad-hoc selected microorganisms with the aim to control diseases caused by xylem inhabiting pathogens such as *Xylella fastidiosa* or modify olive plant physiology and growth.

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Transcriptome profiling of two coffee varieties in response to infection with an endemic *X. fastidiosa* subsp. *fastidiosa* strain from Costa Rica

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X. fastidiosa subsp. *fastidiosa* is endemic in Costa Rica. Since the 1990s, the presence, the broad genetic diversity of the isolates, and dissemination of the bacteria have been confirmed throughout the different coffee-producing regions of the country. Although the bacteria is prevalent and has great potential for disease, most of the colonized coffee plants are asymptomatic or to a major extent show mild symptoms that relate to "crespera" disease. The presence of *X. fastidiosa* does not represent a serious threat to the coffee industry in Costa Rica, a different scenario to other known crops. To understand the host-pathogen interaction of *X. fastidiosa* with coffee plants, we performed transcriptomic analysis on two varieties of coffee: Catuaí, a common variety used throughout the country, and CR95, a variety that has shown resistance to fungal pathogens. We assessed plant response to *X. fastidiosa* seven days post-infection. In vitro grown six-month-old coffee plants were inoculated with *X. fastidiosa* subsp. *fastidiosa* (ST33), previously isolated from coffee, or with PBS in case of the control groups (five plants per group). Seven days post-infection the presence of the bacteria was determined by qPCR. In addition, RNA sequencing was performed for each infected and control plant. Our results show that CR95 plants have a lower overall bacterial load seven days post-infection than Catuaí. The Principal component analysis (PCA) clustered healthy and infected replicates separately. CR95 showed twice differentially expressed genes than Catuaí. GO enrichment and network analyses revealed the involvement of several metabolic pathways in response to the exposure to the bacteria. Genes related to plant recognition and defense response were also identified. In conclusion, coffee plants recognize the presence of *X. fastidiosa* but do not induce a strong defense response, a window of opportunity that may favor plant colonization and persistence.

Keywords: ARN-seq, Trancriptomics, coffee

Study of the complementation of *Xylella fastidiosa* causing CVC with a functional polygalacturonase enzyme and its impact on bacterial physiology

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Xylella fastidiosa is a phytopathogen restricted to the xylem of crops such as citrus and grapevine, causing citrus variegated chlorosis (CVC) and Pierce's disease (PD). Comparative genomics studies between the subspecies *fastidiosa* (Xf-PD) and *pauca* (Xf-CVC), it was observed that the precursor gene of the enzyme polygalacturonase (pglA) in Xf-CVC presents a frameshift that generates a non-functional protein. PglA degrades the pectins on the cell wall, allowing the movement of the pathogen. It suggests that the mutation of pglA in Xf-CVC may lead to the slower colonization observed in citrus or prevent the triggering of the plant immune system by DAMPs. To verify whether the expression of pglA confers greater virulence or absence of symptoms, Xf-CVC was complemented with the functional gene of Xf-PD, which was cloned into vector pXF20 and the complementation was confirmed by conventional PCR and restriction enzymes. The complemented bacteria was inoculated in sweet orange and after seven months they still had no symptoms, suggesting that the presence of pglA does not accelerate symptoms in citrus, and probably, the degradation of pectin leads to the activation of the immune system. Due to its fastidious behavior, further evaluations are necessary. To prove in vitro complementation and in vivo expression, a specific antibody against pglA was produced. For this, pglA-PD was cloned into pET28a vector, transformed into *E. coli* Rosetta and induced in a heterologous system. The protein was expressed in the insoluble fraction of the protein extract, isolated via SDS-PAGE and the antibody was synthesized for validation.

Keywords: *Xylella fastidiosa*, Citrus Variegated Chlorosis, Polygalacturonase, Pectin

The new update of the European Food Safety Authority database of *Xylella* spp. host plant species

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Following the *X. fastidiosa* oubreak in Italy in 2013, EFSA was requested by the European Commission to provide scientific assistance on this plant pathogenic bacterium. EFSA published several scientific opinions on this topic, all included in the EFSA Journal virtual issue on *X. fastidiosa* (https://efsa.onlinelibrary.wiley.com/doi/toc/10.1002/(ISSN)1831-4732.XylellaVI). EFSA also released a database of host plant species of *Xylella* spp. that was first published in September 2018. A systematic literature review allowed the compilation of lists of host plant species, along with collection of information on infection conditions, geographic locations, pathogen taxonomy and tolerant/resistant response of host plants. The update published in April 2020 was completed following a comprehensive search of the latest scientific literature and EUROPHYT outbreaks notifications. Thirty-seven new plant species were identified as hosts of *X. fastidiosa*. Most of them were naturally infected and were found in EU countries (France, Italy, Portugal, and Spain) and non-EU countries (USA and Iran). Six new Sequence Types (STs) were identified in Brazil, Italy, and the USA. The list now comprises 343 host plant species detected by two highly reliable detection techniques, with a total of 595 host plant species regardless of the detection method. The raw data were published in the Zenodo platform in the EFSA Knowledge Junction community (https://doi.org/10.5281/zenodo.1339343) and interactive reports are available in the freely accessible Microstrategy platform (https://www.efsa.europa.eu/en/microstrategy/xylella). Since 2021, this systematic approach will be repeated regularly to keep up-to-date the host plant database and a new release is expected in June 2021. The EFSA database of *Xylella* spp. host plant species represents a key tool for researchers, risk assessment and risk management.

Attempts for improving protocol to phenotype olive cultivars responses to *Xylella fastidiosa* infections

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The incubation period of *Xylella fastidiosa* (Xf) in olive could last as long as >1 year, even in the case of the highly pathogenic Xf subsp. *pauca*, ST53. Such long latency period is a major constrain when performing large screening program for searching potential resistant olive genotypes. In this work, we compared the incubation period of the infections on 9 grafted and self-rooted plants of the cultivar Cellina di Nardò. Plants were inoculated by caging on each plant, 10 specimens of *Philaneus spumarius* previously confined for bacterial acquisition on field-infected trees. Plants were maintained under controlled conditions and monthly sampled. One-month post-inoculation (pi), Xf was detected in 1 grafted and 1 self-rooted plant. Six-months pi, 5 self-rooted plants and all 9 grafted plants were positive. One-year pi, 7 out 9 self-rooted plants tested positive, while 2 remained negative.

Initial symptoms (defoliation of the apical shoots) started to appear 5-months pi on 1 grafted plant, and then progressed with all grafted plants exhibiting symptoms within 8 months pi.

Symptoms on the infected self-rooted plants were delayed compared to the grafted plants: initial symptoms appeared 8-months pi on 2 plants, and only at 15-months pi the remaining infected plants showed symptoms. The delayed appearance of the symptoms (approx. 6 months) correlated with a lower symptom severity on self-rooted plants. Interestingly, the roots of grafted plants harboured a higher bacterial population size than self-rooted plants.

The results of this experiment showed that: (i) vector-mediated transmission is an efficient mean for the inoculation of olive plants; (ii) the use of grafted plants may reduce the latency period in olive; (iii) the bacterium in olives successfully colonize the roots. Nevertheless, the differential plant response recorded in our experiment, once validated in field conditions, may provide useful indications when realizing new olive plantations in the infected area.

Keywords: rooted and grafted olive plants, symptoms

Genetic characterization of different *Vitis* species using SSR markers associated with the resistance gene PdR1

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Pierce's disease caused by the bacteria *Xylella fastidiosa* affects several crops including grapevine. Regarding bibliographic references, several molecular markers associated with the PdR1 gene for resistance to *Xylella fastidiosa* have been described in the specie *Vitis arizonica*. Six microsatellite markers (Short Sequence Repeats, SSR) flanking the PdR1 gene were selected for evaluation in a large set of grapevine varieties from different national sources and compared with the tolerant variety. The plant material selected for the study includes American, *vinifera* and wild species no tested so far. SSRs analysis showed that allelic patterns obtained between presumably susceptible varieties and the tolerant *V. arizonica* variety differed. Subsequent principal component analysis (PCA) showed clusters defined at the species level but not at the varietal level. It is also observed that the *V. arizonica* variety tested shows an allelic pattern of heterozygosity similar to the resistant variety described in the literature. Knowing the susceptibility of the *vinifera*, we corroborate this trend in new varieties relevant to wine growing in the Basque Country as well as for other known materials. This study will be complemented with the evaluation of the varietal response to infection at phenotypic and molecular level.

Search for crop species immune to *Xylella fastidiosa* subsp. *pauca*, ST53

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Up to date 35 different host species have been reported susceptible to *Xylella fastidiosa*, subsp. *pauca*, ST53 (Xfp), in the Apulia region (southern Italy). Xfp is genetically related to coffee strains of the subsp. *pauca*, particularly aggressive on olives, the predominant crop species in the area, and on other species (i.e., oleander, *Acacia* spp, *Polygala myrtifolia*). On the other hand, our previous investigations demonstrated that this genotype was not able to infect *Vitis* spp., several species of *Citrus* and the *Prunus* hybrid GF677. In the last 3 years, we extended the investigations by performing a series of artificial inoculations on different species, with the aim of identifying additional immune species. These included *Pyrus communis*, *Juglans regia*, *Ceratonia siliqua*, *Mespilus germanica*, *Diospyros kaki*, *Citrus limon*, *Punica granatum*, *Persea americana*. At least 10 plants for each species were needle-inoculated in 2017, along with susceptible controls (periwinkle and olive). Vector-mediated transmission was also used for *P. americana*, by caging the plants with Xfp-infected specimen of *Philaenus spumarius*. Inoculated plants were periodically inspected and sampled to assess bacterial multiplication and host colonization. Overall, the diagnostic tests performed during the 3 years post-inoculation did not reveal the presence of the bacterium in the tissues above (>10cm) the inoculation points of any of the inoculated plants. Detection occurred only when leaf petioles from the inoculation points were tested. Similarly, no positive detections occurred in the plants of *P. americana* exposed to infected insects. In addition, for *C. limon*, *P. granatum*, *P. communis* and *C. siliqua* surveys conducted in these last 3 years in the heavily infected area did not reveal the occurrence of natural infected plants. Overall, the data suggest that these species do not support the multiplication and the spread of the bacterium. However, even if for *P. americana* needle-inoculations were complemented with vector transmissions, for the remaining species mechanical inoculations should be integrated by vector-mediated transmission tests for conclusive assessment of their immunity.

Keywords: no host, immunity, *Xylella*

Evaluation of xylem vascular occlusions in olive cultivars infected with *Xylella fastidiosa*

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Olive Quick Decline Syndrome (OQDS), caused by the vector transmitted bacterium *Xylella fastidiosa* (Xf), is the most devastating olive disease in south Apulia. Studies in the Pierce's Disease grapevine pathosystem indicate that the biofilm-embedded bacteria engulf the xylem conduits thus eliciting the formation of tylose-like structures and gums aiming to isolate the pathogen. We attempted to evaluate the presence of vascular occlusions in diverse Xf-infected olive cultivars and to assess their nature by fluorescence and transmission electron microscopy (TEM). The observations were performed on healthy and Xf-infected twigs of the Cellina di Nardò, Leccino and FS17 cultivars. The percentages of completely occluded vessels were visually determined by fluorescence microscopy on hand made cross-sections of one-year-old olive twigs from artificially pin-pricked infected and mock-inoculated plants grown under controlled conditions. Occlusions were negligible in healthy plants, while significantly higher in Xf-infected plants reaching the highest value (9.65%) in the susceptible cultivar Cellina di Nardò, while these were 6.81% and 1.33% in the Leccino and FS17, respectively. In addition, the distribution of occluded vessels was not uniform among individual twigs of the same cultivars, ranging from 1% to 34% in Cellina di Nardò, 0.044% to 14% in Leccino and 1.09% to 1.53% in FS17. Tissue portions in which occluded vessels were found by fluorescence microscopy, were embedded in resin to be observed by TEM. Thin sections observations showed that the olive response to the Xf infection does not include the production of tylose-like structures. Conversely, the obstructions we seen by fluorescence microscopy are due to bacterial cell aggregates embedded in a hard matrix. Indeed, as observed by the IAWA (International Association of Wood Anatomists) bulletins for taxonomic identification of hardwood species, olive, differently from grapevine, has very small, half-bordered vessel ray and vessel-parenchyma pits which make unlikely the tyloses development.

Keywords: vascular occlusions, *Xylella fastidiosa*, transmission electron microscopy

Pathogenicity and systemic colonization of Spanish strains of *Xylella fastidiosa* subsp. *multiplex* and *pauca* on olive under controlled conditions

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Since the first detection of *Xylella fastidiosa* (Xf) in the Balearic Islands and Alicante province (mainland Spain), research has provided new insights on genetic diversity and host range of the strains present in the different Spanish outbreaks. The high genetic diversity of Xf in Spain (three subspecies and four STs) and the high host range (over 30) in total implies a risk for several host of agronomic interest if these genotypes spread to areas where they are not present yet. The main goal of this study was to evaluate the pathogenicity and the systemic colonization of four Spanish strains on three olive cultivars representative of the most widely grown in Spain. Two experiments are being conducted in a confined growth chamber (26ºC day/24ºC night), where 10-month-old self-rooted micro-propagated olive cultivars (Arbequina, Hojiblanca and Picual) were inoculated with the strains XYL1961 of Xf subsp. *pauca* ST80 and the Italian strain De Donno of Xf subsp. *pauca* ST53 (Control), strain XYL1966 of Xf subsp. *multiplex* ST81 isolated from olive trees in the Balearic Islands, and the strains IVIA5901 and ESVL of Xf subsp. *multiplex* ST6 isolated from almond trees in the Guadalest Valley (Alicante). Leaf samplings were carried out at the inoculation point (IP), and at 5, 10, 15 and 20 cm above the IP, at 1, 3, 6, 12 and 18 months post-inoculation (mpi), respectively. Furthermore, destructive sampling of different sections of the main stem was performed at 9 mpi at the same levels of leaf sampling. qPCR tests were used to determine the presence and systemic colonization of the bacterium. After 2 years, no clear or specific symptoms associated to Xf infection has been observed. Our results show that all the strains of Xf subsp. *multiplex* were detected in the leaf petioles at the IP and 5 cm above it although at low frequency (<33%) but were not detected after 6 mpi on samples at 15-cm above the IP. In parallel, the strains were detected in the most upper part of the main stem, although in a very low proportion of samples (8-42% depending on the strain) and with low inoculum densities (Cq > 34) indicating that the strains had some trouble colonizing the different olive genotypes. On the contrary, Xf subsp. *pauca* strains could be detected on all leaf petiole samples even up to 20 cm over the IP, although the frequency of positive samples and bacterial inoculum decreased drastically from the IP. When sampling the main stem, results indicated that the Spanish strain of Xf subsp. *pauca* ST80 colonized a higher number of plants and multiplied better than the Italian strain of Xf subsp. *pauca* ST53 which indicated the risk that this strain may represent for olive. No clear differences could be observed among the patterns of infection of the different Xf strains on the three olive cultivars tested.

Acknowledgement

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The Arabidopsis immune receptor EFR increases resistance to *Xanthomonas* and *Xylella* in transgenic sweet orange

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Previous work showed that heterologous expression of the EFR pattern recognition receptor from *Arabidopsis thaliana*, a leucine-rich repeat receptor kinase recognizing bacterial EF-Tu (or the derived peptides elf18/elf26), provides broad-spectrum resistance against bacterial pathogens in different species. Transgenic sweet orange constitutively expressing AtEFR were generated to access whether this receptor would confer increased anti-bacterial resistance in citrus. After genetic confirmation of transgenic events, we confirmed AtEFR functionality by measuring reactive oxygen species (ROS) production, expression of immune marker genes, activation of MAP kinases and bacterial infection assays with *Xylella fastidiosa* subsp. *pauca* and *Xanthomonas citri* subsp. *citri* in transgenic events. Notably, detached leaves from transgenic plants infiltrated with *X. citri* (the causal agent of citrus canker) showed less symptoms and reduced bacterial population. Similarly, when inoculated with *X. fastidiosa* (the causal agent of CVC - Citrus Variegated Chlorosis) the transgenes perceived the bacteria and somehow restrained its growth through the upper parts of the plants. After 18 months, no or mild symptoms were observed. Altogether, our results in sweet orange indicate that EFR has great potential to improve broad-spectrum anti-bacterial disease resistance in perennial crops such as *Citrus*.

POSTER SESSION:
Epidemiology and modeling of *Xylella fastidiosa* diseases

Title	Presenter	DOI
Environmental model to manage the eradication of almond trees of Alicante (Spain) in the case of *Xylella fastidiosa*	**Cortes Plana JJ,** Dept Tecnología Informática y Computación, University of Alicante (ES)	**https://doi.org/10.5281/zenodo.4679518**
Risk of establishment of Pierce's disease in main wine-producer regions worldwide*	**Giménez-Romero A**, Instituto de Física Interdisciplinar y Sistemas Complejos IFISC (CSIC-UIB), Campus UIB, Palma de Mallorca (ES)	**https://doi.org/10.5281/zenodo.4679503**
Future changes in climate suitability of Europe for *Xyllela fastidiosa*-related diseases induced by ongoing climate change	**Godefroid M,** CBGP, INRAE, CIRAD, IRD, Montpellier SupAgro, Montpellier (FR)	**https://doi.org/10.5281/zenodo.4679511**
The spread of *Xylella fastidiosa* in the south-eastern Iberian Peninsula: combining spatial and regional geographical approaches	**Gutiérrez-Hernández O,** Department of Geography, University of Málaga (ES), Spain	**https://doi.org/10.5281/zenodo.4672357**
A web-based GIS tool for estimating the vulnerability to *Xylella fastidiosa*, at European level	**Kalaitzidis, C,** CIHEAM/Mediterranean Agronomic Institute of Chania, Chania (GR)	**https://doi.org/10.5281/zenodo.4672277**
Model-assisted epidemiological inference and surveillance for *Xylella fastidiosa* in France	**Martinetti D**, INRAE, BioSP, Avignon (FR)	**https://doi.org/10.5281/zenodo.4679499**
Inferring the potential spread of *Xylella fastidiosa* in Great Britain*	**Occhibove F**, UK Centre for Ecology & Hydrology, Wallingford (UK)	**https://doi.org/10.5281/zenodo.4672334**

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Environmental model to manage the eradication of almond trees of Alicante (Spain) in the case of *Xylella fastidiosa*

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Mathematical-computational modelling is a very valuable tool for decision-making and proper management of the protocols used to limit the damage caused by an epidemic in living beings. Biological invasions are a process with four stages: arrival, establishment, spread and concentration. All the processes involved can model on a system of ordinary differential equations (ODE) that describes the evolution of populations confined in compartments according to their states. However, this type of modelling has some shortcomings, for example, not considering local interactions between the individuals that make up the population. In addition, differential equations assume that individuals are homogeneously distributed, and all connected to each other, therefore, it is difficult to access the individual dynamics that can occur. These difficulties can be overcome by discrete models (based on agents or cellular automata). Our compartmental model bases on a time-space representation in the form of a grid in which each cell represents either a tree that can be infected under the influence of the whole ecosystem. The state of the grid is updated over time. As many grids as factors in the entire ecosystem will be considered. The update rules and the definition of the probabilities of infection will be described by Boolean rules and neighbourhood environments in the grids. Our proposal provides suitable modelling to the spread of plant pest and particularly to the propagation of Xf in the almond trees. This approach allows the study of the response of the individual trees according to both the combination of variables of an ecosystem and
their intensity. Another advantage of the model is that it allows easy scaling when the number of characteristics of the ecosystem increases. These encouraging results can guide the modeling of tools to advise the appropriate control.

Keywords: *Xylella fastidiosa*, disease expansion, computational modelling, spacetime framework, neighbourhood, update rules

Future changes in climate suitability of Europe for *Xyllela fastidiosa*-related diseases induced by ongoing climate change

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Global change is currently inducing shifts in the distribution of many species, including harmful pests. In the present study, we addressed the potential future response of *Xylella fastidiosa*-related diseases to ongoing climate change. Using available data documenting the worlwide Xf distribution and bioclimatic data, we fitted correlative species distribution models to depict the climatic niche of harmful Xf-related diseases (e.g. Pierce's disease). We then predicted the potential climatic suitability in Europe for these diseases under future climate conditions simulated by global circulation models considering various future scenario of greenhouse gas emissions. Models predict that some region including economically important agricultural areas (e.g. most of wine-producing regions of Western Europe) might likely experience an important increase in climatic suitability for the pathogen by the period 2040-2060. These results are crucial for European agriculture and, are important for the design of future control and monitoring strategies as well as the implementation of research projects.

The spread of *Xyella fastidiosa* in the south-eastern Iberian Peninsula: combining spatial and regional geographical approaches

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The detection of the bacterium *Xylella fastidiosa* in Europe evidences the risks and potential effects of invasive plant pathogens at the crop, landscape, and regional scales. This study aims to combine spatial and regional geographical approaches. For this purpose, we developed a spatial analysis of the spread of *X. fastidiosa* in the south-eastern Iberian Peninsula, specifically the province of Alicante (Valencian Community, Spain). First, we integrated into a Geographic Information System (GIS) the presence records of *X. fastidiosa* published in an official report submitted by the Valencian Government (Diari Oficial de la Generalitat Valenciana). Second, we applied a set of spatial analysis techniques to study the pathogen's dispersion. Preliminary results show that the progressive spread of *X. fastidiosa* in the south-eastern Iberian Peninsula has a directional distribution conditioned by geography, and the spatial pattern expressed is clustered. These results provide valuable information for monitoring outbreaks of *X. fastidiosa* and the early adoption of control measures based on spatially explicit data. Geography matters and combining spatial and regional geographical approaches is a challenge for future research**.**

A web-based GIS tool for estimating the vulnerability to *Xylella fastidiosa*, at European level

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In the framework of the Xf-ACTORS project and in particular WP8 (Regional risk assessment to anticipate the threat and impacts of Xf diseases), an interactive web-based GIS tool has been implemented, allowing the risk assessment of the *X. fastidiosa* overall suitability across Europe. The tool considers the environmental suitability, including factors such as climate, land cover, soil water content, etc., as well as the stakeholders' knowledge and awareness of the disease (RISK) and the perceived impact that the governance will have in preventing or mitigating the impact of the disease (GOV). Samples from the region of Puglia in Italy and Alicante in Spain, have been used to determine the critical thresholds of the independent variables and derive a quantitative relationship between the values of those variables and the vulnerability value, representing the suitability of the area in question for the disease. A series of 6 questions (3 for the stakeholders' perception and 3 for the governance) are presented to the user, who has an option of 5 answers (strongly agree, agree, neutral, disagree, strongly disagree), which are then used to calculate the values of the RISK and GOV variables. The result of the processing is a map indicating the areas from very high to very low risk. The user has the option of recalculating the results, based on different answers to the questions, hence creating different scenarios of stakeholders' perception and governance ability. Even though the data used to derive the relationship originated from only two areas, the tool appears to identify areas where the disease has been actually detected, as highly vulnerable. Further in-situ data, with a greater geographic distribution, would significantly increase the accuracy of the tool.

Keywords: Hyperspectral, thermal, *Xylella fastidiosa*, nutritional, spread model

Model-assisted epidemiological inference and surveillance for *Xylella fastidiosa* in France

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This communication reviews different results on epidemiological parameters' inference and risk-based surveillance obtained from the modelling of the *Xylella fastidiosa* outbreak in France. Firstly, statistical and mechanistic models have been used to infer plausible values for (or the role of) several epidemiological parameters, such as environmental conditions favoring the establishment of the infection, hidden host compartments, the date and location of introduction of the pest in Corse island, as well as host-to-vector and vector-to-host capacities of disease transmission. Secondly, we will show how the aforementioned models can predict the risk of establishment of the disease in previously uncolonized area, and how the resulting risk maps are exploited to design risk-based surveillance strategies. In terms of surveillance, we will also present a newly proposed desktop approach for passive surveillance based on text mining and web-scraping.

POSTER SESSION:
Vectors biology and control

Title	Presenter	DOI
Electrophysiological and behavioral responses of *Philaenus spumarius* and Neophilaenus campestris females to host plant volatiles	**Anastasaki E**, Benaki Phytopathological Institute, Kifissia (GR)	**https://doi.org/10.5281/zenodo.4680160**
Presence, phenology and seasonal abundance of insects, potential vectors of *Xylella fastidiosa* in Greece	**Antonatos S,** Benaki Phytopathological Institute, Kifissia (GR)	**https://doi.org/10.5281/zenodo.4679786**
First assays on the response of adults of *Philaenus spumarius* (Hemiptera: Aphrophoridae) to different host plants	**Aure CM,** Instituto Valenciano Investigaciones Agrarias; Herrero-Schell J., IVIA (ES)	**https://doi.org/10.5281/zenodo.4680075**
Vibrational communication of *Philaenus spumarius* and insights for vibrational pest control	**Avosani S**, Department of Civil, Environmental and Mechanical Engineering, University of Trento, Italy and Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige (IT)	**https://doi.org/10.5281/zenodo.4679792**
Population genetics of the meadow spittlebug *Philaenus spumarius*, the main insect vector of *Xylella fastidiosa* in Europe	**Biello R,** John Innes Centre, Norwich (UK)	**https://doi.org/10.5281/zenodo.4681171**
Dispersal of *Philaenus spumarius*, vector of *Xylella fastidiosa*, in olive grove and meadow agroecosystems	**Bodino N**, CNR–Istituto per la Protezione Sostenibile delle Piante, Torino (IT)	**https://doi.org/10.5281/zenodo.4670310**
Studies on the competence of potential *Xylella fastidiosa* vectors in the Balearic Islands (Spain)	**Borràs D,** Serveis de Millora Agrària i Pesquera. Laboratory for Plant Health. Government of the Balearic Islands, Palma de Mallorca (ES)	**https://doi.org/10.5281/zenodo.4679754**
Microbial assemblages within *Philaenus spumarius* and their possible role on insect´s reproduction*	**Cameirão C,** Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Bragança (PT)	**https://doi.org/10.5281/zenodo.4680278**
Behavioural ecology of the main vector of *Xylella fastidiosa, Philaenus spumarius*	**Cascone P,** National Research Council of Italy, Institute for Sustainable Plant Protection, Portici (IT)	**https://doi.org/10.5281/zenodo.4680084**

Influence of the temperature on the acquisition efficiency of *Xylella fastidiosa* by *Philaenus spumarius*	**Cavalieri V,** Institute for Sustainable Plant Protection, CNR, Bari, Italy	**https://doi.org/10.5281/zenodo.4680388**
Evaluations of insecticides to reduce transmission of *Xylella fastidiosa* in olives	**Cavalieri V,** Institute for Sustainable Plant Protection, CNR, Bari and Centro di Ricerca, Sperimentazione e Formazione in Agricoltura "Basile Caramia", Locorotondo (IT)	**https://doi.org/10.5281/zenodo.4680951**
Identifying the drivers of abundance of *Philaenus spumarius* in Corsica	**Chartois M,** CBGP, INRAE, CIRAD, IRD, Montpellier SupAgro, Univ. Montpellier, Montferrier-sur-Lez (FR)	**https://doi.org/10.5281/zenodo.4680290**
DNA-barcoding and assessment of the genetic diversity of the *Xylella fastidiosa* vectors in the Balearic Islands*	**Delgado-Serra S,** University of the Balearic Islands (ES)	**https://doi.org/10.5281/zenodo.4682089**
Large scale testing of sticky traps for monitoring spittlebugs in different crops	**Dongiovanni C,** Centro di Ricerca, Sperimentazione e Formazione in Agricoltura "Basile Caramia", Locorotondo (IT)	**https://doi.org/10.5281/zenodo.4680867**
First sharpshooter species proven as vectors of *Xylella fastidiosa* subsp. multiplex in *Prunus salicina* trees in Brazil	**Esteves MB,** Departamento de Entomologia e Acarologia, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo (ESALQ/USP), Piracicaba, (BR)	**https://doi.org/10.5281/zenodo.4679789**
Transmission of *Xylella fastidiosa* subsp. pauca to olive trees by sharpshooters and spittlebugs common in Brazilian orchards*	**Froza JA,** College of Agriculture "Luiz de Queiroz", University of São Paulo, Piracicaba (BR)	**https://doi.org/10.5281/zenodo.4680847**
Population fluctuation of predominant sharpshooters and spittlebugs in olive orchards of Southeastern Brazil	**Froza JA,** College of Agriculture "Luiz de Queiroz", University of São Paulo, Piracicaba (BR)	**https://doi.org/10.5281/zenodo.4680854**
Confirmation of coffee related *Xylella fastidiosa* vectors (Cicadellidae) in Costa Rica	**Garita L,** Universidad de Costa Rica, San José (CR)	**https://doi.org/10.5281/zenodo.4680357**
Genetic diversity and Wolbachia infection of Italian populations of *Philaenus spumarius,* the main vector of *Xylella fastidiosa* in Southern Europe	**Giorgini M,** CNR, Institute for Sustainable Plant Protection, Portici (IT)	**https://doi.org/10.5281/zenodo.4680138**

Genetic diversity and bacterial community of European populations of Philaenus spp. and Neophilaenus spp., insect vectors of *Xylella fastidiosa*	**Kapantaidaki D,** Benaki Phytopathological Institute, Kifissia (GR)	**https://doi.org/10.5281/zenodo.4679782**
Impact of insecticides in the feeding behaviour of *Philaenus spumarius* associated to the transmission of *Xylella fastidiosa*	**Lago C,** Instituto de Ciencias Agrarias (ICA). Consejo Superior de Investigaciones Científicas (CSIC), Madrid (ES)	**https://doi.org/10.5281/zenodo.4670189**
Identification and monitoring of *Xylella fastidiosa* potential vectors on modern olive orchard	**Lamarosa A,** Instituto Politécnico de Beja, Escola Superior Agrária (PT)	**https://doi.org/10.5281/zenodo.4679747**
Understanding host-plant shifting of *Philaenus spumarius* in UK	**Lester K,** Science and Advice for Scottish Agriculture (SASA), Edinburgh (UK)	**https://doi.org/10.5281/zenodo.4679776**
Philaenus spumarius and *Neophilaenus campestris* as efficient insect vectors for *Xylella fastidiosa* in Majorca (Spain)	**López-Mercadal J,** University of Balearic Islands, Palma (ES)	**https://doi.org/10.5281/zenodo.4681106**
Efficiency of different trap types for the monitoring of *Philaenus spumarius*	**Markheiser A,** Julius Kühn-Institut (JKI), Institute for Plant Protection in Fruit Crops and Viticulture, Siebeldingen (DE)	**https://doi.org/10.5281/zenodo.4680197**
Relative efficacy of different colour sticky traps for the capture of vectors of *Xylella fastidiosa*	**Mercadal P,** University of Balearic Islands, Palma (ES)	**https://doi.org/10.5281/zenodo.4680648**
Wanted egg parasitoids: *Ooctonus vulgatus* parasitizes *Philaenus spumarius* in Corsica and is probably widely distributed in Europe	**Mesmin X**, CBGP, INRAE, CIRAD, IRD, Montpellier SupAgro, Univ Montpellier, Montpellier (FR)	**https://doi.org/10.5281/zenodo.4680103**
Vectors of *Xylella fastidiosa* show pronounced habitat preferences in Corsican agricultural landscapes	**Mesmin X,** CBGP, INRAE, CIRAD, IRD, Montpellier SupAgro, Univ Montpellier, Montpellier (FR)	**https://doi.org/10.5281/zenodo.4680115**
Response of *Philaenus spumarius* and *Neophilaenus campestris* to potential semiochemicals	**Nencioni A,** Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence; Institute of BioEconomy, Biology, Agriculture and Food Sciences Department – National Research Council (IT)	**https://doi.org/10.5281/zenodo.4680958**

Attractiveness of different colored sticky traps for spittlebug vectors of *Xylella fastidiosa*	**Nencioni A,** Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence; Institute of BioEconomy, Biology, Agriculture and Food Sciences Department – National Research Council (IT)	**https://doi.org/10.5281/zenodo.4680213**
Detection of *Philaenus* (Hemiptera: Aphrophoridae) DNA in the gut of spiders, using PCR-based gut-content analysis	**Rodrigues I**, Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança and Departamento de Ingeniería Agrária, Universidad de Léon (ES)	**https://doi.org/10.5281/zenodo.4680701**
Olfactory behavior of *Philaenus spumarius* (Hemiptera: Aphrophoridae) to two naturally occurring volatile compounds on almond, olive, and vine leaves	**Rodrigues I,** Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança and Departamento de Ingeniería Agrária, Universidad de Léon (ES)	**https://doi.org/10.5281/zenodo.4680822**
A web-interface database for the identification of vectors of *Xylella fastidiosa* in Europe	**Streito J-C,** CBGP, Univ. Montpellier, CIRAD, INRAE, IRD, Montpellier SupAgro, Montpellier (FR)	**https://doi.org/10.5281/zenodo.4680659**
Presence, diversity and seasonal fluctuation of *Xylella fastidiosa* potential vectors and other Auchenorrhyncha (Hemiptera) in olive agroecosystems with different management systems	**Tsagkarakis A,** Agricultural University of Athens, Athens (GR)	**https://doi.org/10.5281/zenodo.4679768**

**abstracts selected through the Young researchers' initiative are reported in the oral presentations section only.*

Electrophysiological and behavioral responses of *Philaenus spumarius* and *Neophilaenus campestris* females to host plant volatiles

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The spittlebugs, *Philaenus spumarius* and *Neophilaenus campestris* (Hemiptera: *Aphrophoridae*) are xylem-feeder insects that have been identified as main vectors of *Xylella fastidiosa* in Europe. In the present study we aimed to identify volatile organic compounds (VOCs) that may act as semiochemicals for these species. We collected VOCs from *Olea europaea* and *Polygala myrtifolia*, highly susceptible plant species to *X. fastidiosa*, *Pinus halepensis* a common plant where *N. campestris* is found during summer, and from host plant species that are used as cover crops or exist as natural vegetation in olive orchards like, *Cistus creticus*, *Medicago sativa*, *Cynodon dactylon*, *Festuca arundinacea*, *Apium graveolens* and *Petroselinum crispum* using dynamic headspace technique. We tested the response of adults' antennae on those blends with Gas Chromatography-Electro-Antennographical Detection (GC-EAD). The chemical profile of *C. creticus* and *P. halepensis* was rich in terpenes, alcohols, aldehydes, and esters. As for *P. myrtifolia*, the volatile profile consisted majorly of alkanes and their substitutes. In the volatile profile of *M. sativa*, *C. dactylon* and *F. arundinacea*, common volatile compounds were detected. *Petroselinum crispum* and *Apium graveolens* chemical profile was dominated by terpenes. Numerous compounds elicited EAD responses for both species. α–pinene was present in all tested plants and found to stimulate antennae of female *P. spumarius* in five plants. In addition, antennae of *P. spumarius* females responded in camphor, limonene, 4-methyl octane and sabinene. These compounds were found in the volatile profile of at least 5 out of 8 examined plant species. The VOCs that triggered the most frequent EAD responses in *P. spumarius* females' antennae were selected for olfactometer behavioral bioassays depending on availability of synthetic compounds. Behavioral studies with authentic samples, namely (-)-α-pinene, (+)-α-pinene, sabinene, (-)-S-limonene and (1R)-(+)-camphor showed a significant attraction to camphor for *P. spumarius* females.

Keywords: GC-EAD; olfactometer; VOCs

Presence, phenology and seasonal abundance of insects, potential vectors of *Xylella fastidiosa* in Greece

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Xylella fastidiosa is a xylem inhabiting bacterium which is exclusively transmitted by xylem sap feeding insects. Extensive samplings were conducted in 28 olive orchards in Greece in 2017 and 2018 aiming to identify the presence of *X. fastidiosa* potential vectors. Moreover, in 2018 and 2019, the phenology and seasonal abundance of the most frequently occurred species in three different geographical regions of Greece were studied. Our surveys revealed the presence of five species belonging to the family *Aphrophoridae* and one species of the family *Cercopidae*. The most frequently observed spittlebugs were *Philaenus spumarius* and *Neophilaenus campestris*. *Philaenus spumarius* was found in 13 olive orchards distributed in nine regional units and *N. campestris* was observed in five olive orchards distributed in five regional units. Other xylem feeders recorded were *P. signatus*, *Lepyronia coleoptrata*, *Aphrophora alni* and *Cercopis sanguinolenta*. Nymphs of *P. spumarius* and *N. campestris* were observed between early March and mid-May depending on geographic location and year. Adults were present during two periods each year, in spring and in autumn-early winter. During summer months, spittlebugs were totally absent from olive orchards. The knowledge of the presence and seasonal abundance of those insects is crucial for the implementation of a successful control strategy in case of *X. fastidiosa* outbreaks.

First assays on the response of adults of *Philaenus spumarius* (Hemiptera: *Aphrophoridae*) to different host plants

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Spittlebugs are responsible for the spread of *Xylella fastidiosa* in several Europan countries. This bacterium (*X. fastidiosa* subspecies *multiplex*) was found attacking almond trees in the Valencian Community (Eastern Spain) in 2017, producing the "Almond Leaf Scorch Disease". Since then, *Philaenus spumarius* has been identified as the main insect species involved in transmission between plants. One key factor in understanding the epidemiology of the bacterium is to determine the host plant feeding preference of its insect vectors. One way to stablish this preference is studying the attraction of the vectors to the different plants, depending on the emitted volatile compounds. In this work, we are analyzing the attraction of *P. spumarius* to several host plants by using a Y-tube olfactometer. Individuals of *P. spumarius* came from a greeenhouse, where we reared the insects in semi-field conditions, or directly from the field. Common sowthistle (*Sonchus oleraceus* L.) has been used as a preferred host plant of the insect. Therefore bermuda grass (*Cynodon dactylon* (L.) Pers.), olive (*Olea europea* L., var Arbequina) and citrus (*Citrus sinensis* (L.) Osbeck, var Pineapple) have been compared against it. Besides activated common sowthistle (a plant previously attacked by insects) has been tested. The results have shown that: i) common sowthistle was preferred over no plant, ii) an activated common sowthistle was preferred over a not-activated plant, iii) common sowthistle was preferred over bermuda grass, iv) common sowthistle was preferred over citrus, and v) common sowthistle was similarly prefered to olive. In some cases, it seems that responses to plants depends on insect sex.

The study is ongoing and more tests with *P. spumarius*, as well as the analysis of *Neophilaenus campestris*, are being carried out.

Vibrational communication of *Philaenus spumarius* and insights for vibrational pest control

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Bio-assays were conducted to investigate the mating behavior and associated vibrational signals of the *Xylella fastidiosa* vector, *Philaenus spumarius*. After characterization of vibrations involved in pair formation, studies were conducted to assess whether female receptivity to mating is correlated to ovarian development. It was observed that females emitted mating signals from the end of the summer onward, and that signaling activity and responsiveness to mating were correlated with ovarian development. In addition, to evaluate whether a vibrational stimulus could disrupt mating, pairs were treated with a continuous broad-band noise with a frequency range of 150-1200 Hz. Although both females and males emitted mating signals during the treatment, the noise could disrupt the pair formation process, likely by preventing mate finding. Further research on larger scale should be conducted to develop future control mating disruption techniques, which should be applied at the end of the summer, when both sexes are sexually mature.

Keywords: Biotremology, vector behavior, physiology

Population genetics of the meadow spittlebug *Philaenus spumarius*, the main insect vector of *Xylella fastidiosa* in Europe

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Sap-feeding insects are known vectors of plant viruses and groups of economically important bacterial plant pathogens, such as *Xylella fastidiosa*. The bacterium *X. fastidiosa* was recently detected in Europe and is responsible for the destruction of entire ancient olive groves in Italy. *X. fastidiosa* is transmitted by xylem-feeding insects, including the meadow spittlebug *Philaenus spumarius* (Linnaeus, 1758) (Hemiptera: *Aphrophoridae*), which is the main vector of *X. fastidiosa* in Europe. *P. spumarius* is prevalent across Europe, including the United Kingdom, and the insect has a broad plant host range that enables it to vector *X. fastidiosa* to a diverse range of plant species. However, *P. spumarius* has been predominantly studied at the species level. To investigate the genetic structure of UK populations and assess migration patterns of this species, we first assembled its 2.7 Gb genome. In addition, we sampled *P. spumarius* populations at six localities in the UK (Norfolk, Kent, Sussex, Yorkshire, Wales and Scotland) and re-sequenced the genomes of 116 individuals. Across Europe, *P. spumarius* is split into three divergent mitochondrial lineages (haplogroups), of which two are found in the UK – one in the north and one in the south – whereas the third is predominantly found in southern Europe (e.g., Italy). SNP data from whole-genome sequencing showed a weak genetic structure among the UK populations in support of isolation by geographical distance and evidence of admixture between the two mitochondrial lineages. Furthermore, we did not find a clear clustering of insect genotype by host plant species, indicating that *P. spumarius* is probably a true generalist. We are exploring this further by generating a chromosome-level assembly of *P. spumarius* and using this assembly to reassess the UK *P. spumarius* population structure. Furthermore, we are collaborating with colleagues globally to examine the *P. spumarius* populations by re-sequencing genomes of ±200 individuals from populations across Europe, the USA and New Zealand.

Dispersal of *Philaenus spumarius*, vector of *Xylella fastidiosa*, in olive grove and meadow agroecosystems

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The meadow spittlebug *Philaenus spumarius* plays the major role in the spread of *X. fastidiosa* subsp. *pauca* ST53 on olives in the Apulia region (Italy). Despite the vector's movement is a key component of *X. fastidiosa* epidemiology, little information is available. Dispersal capabilities of the meadow spittlebug were hence investigated by mark-release-recapture (MRR) experiments carried out from May to October in olive grove and meadow, in the Apulia and the Piedmont regions (Italy), respectively. Field-collected adults of *P. spumarius* were marked with an aqueous solution of albumin (10%) and released at a single point in the centre of the experimental area. A total of ≈6500 and ≈3000 spittlebugs were released during Apulia and Piedmont experiments, respectively. Spittlebugs were recaptured with sweep net (10–250 m distance from release point) every two or three days up to 17 days after the release. Marked insects were identified via an indirect ELISA. The dispersal capacity of *P. spumarius* adults was described by estimating the dispersal kernel, i.e. the probability density function describing the distribution of the recapture locations of insects relative to the release point. Spittlebugs were recaptured up to 155 meters and 200 m in Apulia and Piedmont, with 50% of total recaptures within 30 m and 40 m from the release points, respectively. Results showed a high variability in the estimated median distance from the release point, ranging from 26 m (Apulia) to 35 m (Piedmont) per day, and from 374 m (Apulia) to 507 m (Piedmont) for adult life-long dispersion. Estimated spread parameters of *P. spumarius* are fundamental to model *X. fastidiosa* spread in Apulia and other European foci. Further research is needed to investigate the influence of insect physiological status and environmental factors on movement and directionality of *P. spumarius*.

Keywords: Vector dispersal, Xf spread, mark-release-recapture

Studies on the competence of potential *Xylella fastidiosa* vectors in the Balearic Islands (Spain)

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Since *Xylella fastidiosa* (Xf) was first detected in Majorca (Balearic Islands) in October 2016, four sequence types (ST) have been described in the Balearic Islands infecting 28 host species. Xf subsp. *multiplex* (ST 81) is found in Majorca and Minorca and Xf subsp. *pauca* (ST 80) in Ibiza, whereas Xf subspecies *fastidiosa* (ST 1) and Xf *multiplex* (ST7) are only present in Majorca. The bacterium is naturally transmitted by xylem "specialist" insects belonging to the suborder *Auchenorrhyncha* (Hemiptera). In Italy, *Philaenus spumarius* (Hemiptera: *Aphrophoridae*) has been described as main vector of CoDiRO strain (Xf subsp. *pauca*) in olive orchards, even though recently *P. italosignus* and *Neophilaenus campestris* have also been shown to transmit Xf. Studies performed in different orchards from the Balearic Islands revealed that *P. spumarius* together with *N. campestris* were the most abundant *Aphrophoridae* species, being the main candidates for Xf transmission. Transmission tests with *P. spumarius* and *N. campestris* were performed to determine the capacity of local populations to transmit Xf subspecies under semi-field conditions (greenhouse). Xf subsp *fastidiosa* was transmitted to healthy vines by infected *P. spumarius* that previously had fed on infected vines and from almond to almond. On the other hand, in crossed-transmission tests *P. spumarius* was able to transmit Xf subsp. *fastidiosa* from infected almonds to healthy grapevines and from infected grapevines to almond, and Xf subsp *multiplex* from wild olive tree to almond trees. Preliminary research has shown that *N. campestris* can transmit Xf subsp *fastidiosa* vine to vine.

Behavioural ecology of the main vector of *Xylella fastidiosa, Philaenus spumarius*

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The spread of *Xylella fastidiosa* in olive orchards could be hampered only by an integrated approach. Among the possible strategies, there is the manipulation of the behaviour of the main vector of *X. fastidiosa*, the spittlebug *Philaenus spumarius* L. (Hemiptera, *Aphrophoridae*) through the characterization of semiochemicals involved in plant location. Currently, no information are available about the possible compounds regulating the passage of this vector from the grassy cover to olive trees. To fill this gap of knowledge we studied the response of *P. spumarius* towards resistant (Leccino, FS-17) and susceptible (Ogliarola, Frantoio and Rotondella) olive tree varieties in behavioural bioassay. We recorded a different response in relation to olive variety and sex of individuals. Males were not attracted neither by olive trees, regardless the variety tested, nor by clean air. Conversely, females were attracted by susceptible olive varieties, and repelled by FS-17. The characterization and quantification of Volatile Organic Compounds (VOC) released by tested olive varieties, support the female response recorded in the behavioural bioassay. We also tested three ornamental plants, lavender, geranium and citronella, and their relative Essential Oils (EOs) that could alter the behaviour of *P. spumarius*. A differential response in relation to adult sex was recorded in all tests. Plants elicited responses that also varied upon the plant species and the distance from the odour source. Both lavender and geranium repelled females at any distance range whilst males were attracted by geranium and repelled by citronella from a long distance. At close range, lavender and citronella were repellent for females and males, respectively. Only males were distinctly attracted by lavender EO. Our results contribute to the development of innovative tools based on the displacement of *X. fastidiosa* vector from olive tree orchards.

Influence of the temperature on the acquisition efficiency of *Xylella fastidiosa* by *Philaenus spumarius*

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Given the wide range of latitudes at which the bacterium *Xylella fastidiosa* (Xf), susceptible host plants and insect vectors may coexists, we have investigated the effect of different range of temperatures on the capability of *Philaenus spumarius*, the predominant insect vector of Xf in Europe, to acquire and transmit Xf from olive to olive. Plants cv Cellina di Nardò infected with Xf susbsp. *pauca* ST53 were exposed at the following conditions: 38-32°C; 36-28°C; 32-24°C; 28-20°C; 20-14°C. More specifically, to simulate day/night conditions, in each treatment, plants were daily exposed for 10h at the highest temperature, then for 14h at the lowest one. Infected source plants were pre-conditioned at the same temperatures either for 3 or 8 days before starting the experiments.

After an acquisition access period (AAP) of 3 days, insects were moved to receptor plants maintained at the same temperatures of the correspondent AAP. Insect mortality was recorded after both AAP and IAP, whereas the acquisition of Xf was estimated by qPCR on individual insects tested after the IAP. As the experiment is still ongoing, herein we report only the data on the AAP. Briefly, mortality reached average values of 99-100% when insects were caged on the plants maintained at 38°C; it dropped to approx. 60% at 36°C, and to 20-30% at 28°C and 32°C, respectively, while at 20°C it was less than 10%.

The acquisition rate could not be estimated for the highest temperature (due to the high mortality). Acquisition was lower at 36°C (about 9%), compared to the other tested temperatures (about 35%). Only at the highest temperatures, pre-conditioning the plants for 8 days reduced the AAP efficiency compared to 3 days pre-conditioning, suggesting an effect of the high temperature on the bacterium in the host plant. Although limited to the AAP, these results demonstrate a significant impact of high temperatures (> 32°C) on insect survival and Xf acquisition efficiency.

Evaluations of insecticides to reduce transmission of *Xylella fastidiosa* in olives

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The efficacy of several natural and synthetic compounds to suppress populations of *Philaenus spumarius*, the predominant EU vector of *Xylella fastidiosa*, has been investigated in the past recent years. As for many other vector-borne diseases, control strategies aim also to reduce the feeding events and therefore the chances of transmission. In this context, we evaluated the use of different substances for reducing the rate of bacterial transmission in olives either under filed conditions or confined conditions in cages. In fields located in the infected area of Apulia (southern Italy), we tested the use of kaolin. Healthy plants were sprayed for three consecutive years on a calendar basis with kaolin and imidacloprid (control product). Results showed that neither kaolin and imidacloprid protected the plants from the infections, however for both treatments a delay in the progression of the infections and symptoms severity was recorded compared to the untreated control plants. When kaolin was applied in cages, results were similar, with infections being lower for the treated plants than for the untreated plants. Chemical products tested included imidacloprid, acetamiprid, deltamethrin, phosmet. Experiments conducted in cages were set up using *Xylella*-infected spittlebugs (apprx. 15% of qPCR-positive specimens). Insects were released in the cages after 3, 7 and 15 days after treatment (DAT) and maintained for 3 days for the inoculation access period. High mortality rates were recorded with all insecticides except that for phosmet (inconsistent results). At 3 DAT, no infections were recorded on the seedlings sprayed with neonicotinoids and deltamethrin. Low or no infections were recorded with neonicotinoids and deltamethrin at 7 and 15 DAT, while rates of infections were similar for phosmet-sprayed and untreated controls.

Identifying the drivers of abundance of *Philaenus spumarius* in Corsica

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Philaenus spumarius (Hemiptera: *Aphrophoridae*) is a major vector of *Xylella fastidiosa* (Xf) in Europe. However, the drivers influencing its abundance are still poorly known. In Corsica from 2018 to 2020, we monitored the abundance of *P. spumarius* three times a year in 64 permanent plots distributed along vegetation and altitudinal (0-600m) gradients. The vegetation gradient ranged from shrubland to oak forest and accounted for the density of *Cistus monspeliensis* on which *P. spumarius* usually develops and feed in Corsica. Abundance of *P. spumarius* was estimated through visual counting of foams and sweep netting of adults. Both vegetation composition and climate descriptors were recorded at each sampling location. We used partial least squares path modelling and generalized linear mixed models to unravel the relationships between vegetation / climate descriptors and *P. spumarius* abundance. *Philaenus spumarius* abundance significantly decreases with increasing average temperature and humidity. Abundance of *P. spumarius* was significantly and positively correlated with *C. monspeliensis* density, confirming difference in host plant preference between Corsica and mainland France. A significant effect of vegetation structure was also found with forests hosting lower *P. spumarius* abundance. The strong association observed between *P. spumarius* and *C. monspeliensis* suggests that Xf expansion may have been facilitated by firebreaks or disturbed habitats where C. monspeliensis is abundant as an early colonizer. Managing *C. monspeliensis* in the vicinity of agricultural areas may constitute an essential component of prophylaxis against Xf in Corsica.

Keywords: spittlebug, insect vector, *Auchenorryncha*

Large scale testing of sticky traps for monitoring spittlebugs in different crops

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Monitoring spittlebugs has become crucial for the implementation of preventive measures against the spread of *Xylella fastidiosa* in Europe. So far, sweep nets are commonly used to collect spittlebugs from ground vegetation and canopies of trees or shrubs. From 2018 to 2020, we conducted several field experiments for testing the effectiveness of sticky traps as tool for monitoring insect populations in different crops. We evaluated different coloured sticky traps, compared captures on sticky traps vs sweep nets, and validate their efficiency on different crops (olive, almond and cherry). The inspections of the traps showed that: (i) the most attractive for *Philaenus spumarius* and *Neophilaenus campestris* were those coloured in yellow; (ii) while in olive groves no differences were recorded for the yellow traps with different background, in the almond and cherry orchards, the capture of *P. spumarius* on those with black line-pattern were significantly higher than the other in some of the inspection periods; (iii) leafhoppers were the predominant non-target insects captured on the yellow sticky traps in our experimental fields. Comparison between yellow sticky traps and sweep nets for monitoring *P. spumarius* clearly showed that early in the season, i.e. when the adult emerge, insects were prevalently caught by sweeping net. Later on, the adults captured on traps were constantly higher than by sweep nets, especially in olive groves with medium-high population densities, in almond and cherry orchards. In conditions with low population densities of *P. spumarius*, the two methods showed similar efficiency. Regarding *N. campestris*, no differences were observed between the two sampling methods on olive trees and cherry. While on almond yellow sticky traps were mostly effective than sweep net, prevalently in late summer when leaves fall and sweep net were difficult to use due to the stiffness of the branches. Our results suggest that the use of sweep nets soon after the emergence of the adults coupled with sticky traps for the rest of the adult season, can expedite monitoring programs finalized to the implementation of control measures (i.e. defining the threshold and the best time for insecticide applications).

First sharpshooter species proven as vectors of *Xylella fastidiosa* subsp. *multiplex* in Prunus salicina trees in Brazil

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Plum leaf scald (PLS), caused by the vector-borne bacterium *Xylella fastidiosa*, is a major obstacle to the expansion of plum crops in Brazil. In other affected crops, this pathogen is naturally transmitted mainly by sharpshooter leafhoppers (Hemiptera: *Cicadellidae*: *Cicadellinae*). Despite the importance of PLS, the vector species responsible for bacterial spread in plums remain unknown. This study, therefore, aimed at determining the *X. fastidiosa* transmission ability of three sharpshooter species commonly found in plum orchards in southern and southeastern Brazil: *Macugonalia cavifrons* (Stål), *Macugonalia leucomelas* (Walker), and *Sibovia sagata* (Signoret). After a 72 h acquisition access period on plum trees infected with *X. fastidiosa* subsp. *multiplex* (sequence type 67), four insects of each of the species were transferred for an inoculation access period of 96 h to 10–20 healthy *Prunus salicina* Lindl. Bacterial infection in the plants was verified 7 months after inoculation by polymerase chain reaction. Results showed that the three sharpshooter species were able to transmit *X. fastidiosa* to plums, with mean transmission efficiencies by single insects of 16%, 14%, and 18% for *M. cavifrons*, *M. leucomelas*, and *S. sagata*, respectively. To our knowledge, this study is the first to identify vector species and prove the transmission of a PLS strain of *X. fastidiosa* subsp. *multiplex* by sharpshooters in Brazil.

Population fluctuation of predominant sharpshooters and spittlebugs in olive orchards of Southeastern Brazil

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Xylem-sap feeding leafhoppers, known as sharpshooters (Hemiptera: *Cicadellidae*, *Cicadellinae*) and spittlebugs (Hemiptera: *Cercopoidea*) are vectors of *Xylella fastidiosa*, which causes diseases in various crops. Here we studied the seasonal fluctuation of predominant species of sharpshooters and spittlebugs in olive orchards in four locations in the States of São Paulo (Cabreúva and São Bento do Sapucaí) and Minas Gerais (Maria da Fé and Wenceslau Braz), Brazil, where *X. fastidiosa* is associated with olive quick decline syndrome (OQDS). The insects were trapped by yellow sticky cards (10x30 cm) hanged at 0.8 m above ground in external branches of nine olive trees (spaced 30 m apart) per orchard. Traps were replaced every two weeks from June 2015 to June 2020 and taken to the laboratory for species identification and quantification under a dissecting microscope. Most sharpshooter and spittlebug species showed peaks in population density during months of higher mean temperature and relative humidity (September-February), except for the spittlebug *Clastoptera* sp.1, whose adult population peaked from February to April (late summer and early autumn). The highest population peaks (4-6 insects/trap/period) were observed for *Clastoptera* sp.1 and the sharpshooters *Macugonalia cavifrons* and *Scopogonalia paula* in S. Bento do Sapucaí, and for the spittlebug *Sphenorhina rubra* and the sharpshooter *Erythrogonia phoenicia* in Maria da Fé. Population peaks of *S. paula* and *M. cavifrons* were lower (2-3 insects/trap) and less frequent in Wenceslau Braz, which is located at the highest elevation (1750 m). Late summer and autumn (March-June) peaks (1-2 insects/trap) were observed for the most frequent sharpshooter (*Dilobopterus costalimai*) in Cabreúva, at the lowest elevation (890 m) tested; in this particular location, a single peak (2.5 insects/trap) of the spittlebug *Deois flavopicta* was observed in January/2017. The results suggest that spring, summer and early autumn are periods of higher risk of *X. fastidiosa* spread by vectors in the region.

Confirmation of coffee related *Xylella fastidiosa* vectors (*Cicadellidae*) in Costa Rica

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Xylella fastidiosa is a vector-transmitted plant pathogen of economic importance. In Costa Rica, *X. fastidiosa* is widespread in coffee plantations, positive coffee plants are mostly asymptomatic, or present mild symptoms related to “crespera” disease, as reported previously. Despite this, there is scarce information regarding vector biology in this pathosystem. Past studies have shown that there is great diversity of Hemiptera (*Cicadellidae* and *Clastopteridae*) in coffee production zones affected by *X. fastidiosa* in Costa Rica; these insects represent potential *X. fastidiosa* vectors. However, to our knowledge, confirmation of vector status remains unsettled. In this work, we identified within the population of *Cicadellidae* from a coffee plantation in Carrizal de Alajuela (coffee producing zone in the northern part of Costa Rican central valley), a series of specific species of leafhoppers as true vectors of *X. fastidiosa* related to coffee in Costa Rica. To achieve these results, insects were collected in field conditions, and *Cicadellidae* were identified, classified, and grouped for further analysis. All assays were set up in greenhouse conditions. The ability of these vectors to feed and survive on coffee plants was assessed. Artificial transmission assays, revealed the presence of the bacteria, using qPCR after feeding in at least 6 species of leafhoppers. MLST typing was done to confirm circulating ST. Transmission assays were done on *X. fastidiosa* free coffee plants, detection of the bacteria was achieved in plants as soon as six months, positive plants did not shown symptoms up to 2 years. These findings contribute to the integral understanding of the pathosystem and for the development of mitigation strategies.

Keywords: Vector, leafhoppers, transmission

Genetic diversity and *Wolbachia* infection of Italian populations of *Philaenus spumarius*, the main vector of *Xylella fastidiosa* in southern Europe

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Wolbachia are bacterial symbionts of many insects, acting as reproductive parasites or sometime establishing mutualistic relationships with their hosts. They are able to increase their prevalence within host populations by improving the reproductive fitness of infected females. Their ability to interfere with host reproduction and to be pervasive within insect populations (e.g. by inducing cytoplasmic incompatibility) make *Wolbachia* a promising tool for the biological control of pests and for reducing the spreading of vector-borne pathogens. *Philaenus spumarius* harbors *Wolbachia* but the type of symbiotic relationship is unknown. Further, data on the infection prevalence within host populations are limited, particularly for Italy. We sampled *P. spumarius* populations throughout Italy and correlated the *Wolbachia* infection rate with host genetic diversity and geographic distribution. The haplotype network developed on the COI gene sequences showed the occurrence of the three mitochondrial lineages of *P. spumarius*, namely the eastern-Mediterranean, the western-Mediterranean and the eastern lineage. Each lineage showed a specific geographic distribution. The eastern-Mediterranean haplotypes were prevalent in southern-central Italy, except for Sicily. The western-Mediterranean haplotypes were prevalent in northern Italy. The eastern haplotypes were the less represented and recorded exclusively in the northernmost part of Italy. Admixtures of different lineages were found throughout Italy. PCR with *Wolbachia* specific primers showed that all populations sampled in southern-central Italy were uninfected. Conversely, half of the populations from northern Italy were infected. The infection rate ranged between 9-80% and all three lineages harbored *Wolbachia*, identified within the host reproductive tissues. Analysis of molecular diversity and neutrality test statistics suggested no evidence of *Wolbachia*-induced selective sweep of uninfected haplotypes. *Wolbachia* strains were characterized using the MLST approach and new sequence types were identified within the supergroup B. Our results pave the way for future researches aimed to evaluating the role of *Wolbachia* in *P. spumarius* and its possible exploitation for pest management.

Keywords: COI gene, endosymbiotic bacteria, symbiotic interactions

Genetic diversity and bacterial community of European populations of *Philaenus* spp. and *Neophilaenus* spp., insect vectors of *Xylella fastidiosa*

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The bacterium *Xylella fastidiosa* causing diseases of relevant impact on several plant species worldwide, is mainly transmitted by xylem-feeding insects of the suborder *Auchenorrhyncha*. Given the fact that *X. fastidiosa* poses a serious threat for cultivations in Europe, the knowledge of vectors biology is of great importance. The aim of this study was to explore the genetic diversity (COI, cytb, ITS2) and the bacterial infection status (*Wolbachia*, *Arsenophonus*, *Hamiltonella*, *Cardinium*, *Rickettsia*) of different populations of spittlebug species that are either known vectors of the disease or potential vectors. To this end, populations of different spittlebug species were collected from Greece (*Philaenus spumarius*, *P. signatus* and *Neophilaenus campestris*), Italy and Germany (P*. spumarius, P. italosignus, N. campestris* and *N. lineatus*). Our study indicated different levels of genetic diversity between the insect species. In the Greek populations of *P. spumarius, P. signatus* and *N. campestris*, the ITS2 region was the less polymorphic, while the analyzed fragments of the mitochondrial genes displayed higher genetic diversity. Specimens of *P. spumarius* from Germany and Italy exhibited high genetic variability in the COI gene while *N. campestris* from Germany in the ITS2 region. *N. lineatus* and *P. italosignus* from Germany and Italy, respectively, displayed low genetic variation. Differences were also detected in the bacterial community harbored by the different insect species collected in the abovementioned countries. Most of the Greek populations harbored at least one symbiont. *Rickettsia* was the predominant one, while *Cardinium* was totally absent from all populations. On the other hand, German and Italian specimens displayed lower bacterial diversity, since very few specimens were associated with symbionts. There was no evidence for the presence of *Cardinium* and *Hamiltonella* in any of the four examined insect species. The results of this study will enhance the understanding of the insect-endosymbionts-pathogenic bacteria associations and provide important knowledge to develop strategies aiming to control the spread and transmission of the bacterium.

Keywords: *Xylella fastidiosa*, vectors, genetic diversity, endosymbionts

Impact of insecticides in the feeding behaviour of *Philaenus spumarius* associated to the transmission of *Xylella fastidiosa*

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Philaenus spumarius was identified as the predominant vector involved in the spread of *Xylella fastidiosa* in Europe. Studies on pesticides lethal and sub-lethal effects on insect vectors are essential for the development of sustainable control strategies. Therefore, we studied the effect of six pesticides (acetamiprid, deltamethrin, spinosad, sulfoxaflor, pyrethrin, kaolin) on the survival and feeding behavior of *P. spumarius* when exposed to treated plants (*Sonchus olearaceous*) through the Electrical Penetration Graph (EPG) technique. First, we observed the spittlebug feeding behaviour for 4h on treated plants; second, insect survival was assessed for 4h on treated plants and the feeding behavior of survived individuals was studied on untreated plants. In addition, we evaluated the effect of pyrethrin, acetamiprid, and kaolin on the transmission of *X. fastidiosa* ST53 to *Catharanthus roseus* plants. We carried out "no-choice" transmission tests where insects were caged on plants previously treated with a single compound or "free choice" trials with spittlebugs confined in a cage containing plants treated with the 3 compounds. We found that plants treated with deltamethrin and acetamiprid had a repellent effect (insects escaped) compared to other treatments. These pesticides reduced the duration of Xc (xylem contact), Xi (xylem ingestion) and the time to the first sustained Xi, when compared to control and kaolin. In the second assay, all insects exposed to deltamethrin or acetamiprid died in two hours. No significant differences in the feeding behavior of insects on untreated plants after the exposure to the tested compounds were found. Pyrethrin and acetamiprid reduced the transmission rate of *X. fastidiosa* by *P. spumarius* while kaolin gave similar results as the control untreated plants. Acetamiprid, however failed to reduce the transmission rate under free choice conditions. Overall, our results suggest that pyrethrin and deltamethrin are the most promising compounds to manage the transmission of *X. fastidiosa*.

Identification and Monitoring of *Xylella fastidiosa* potential vectors on modern olive orchard

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The knowledge of *X. fastidiosa* potential vectors is important for control the spread of the disease. Until now, in Europe, have been identified, as potential vectors of *X. fastidiosa*, insects belong to two large families. *Philaenus spumarius* and *Neophilaenus campestris* are the main *X. fastidiosa* vectors from *Aphrophoridae* family. Both are characterized by the presence of a comb-like pattern along the tibias and posterior tarsus. *Cicadella viridis* (L.) (*Cicadellidae* family) was also identified as a *X. fastidiosa* potential vector, which is characterised by the hair presence in posterior tibias. The present work intended to identify and monitor the *X. fastidiosa* potential vectors belong to *Aphrophoridae* and *Cicadellidae* families, in 3 plots of a modern olive orchard in Alentejo (south of Portugal), between August (2019) and June (2020). Insects were captured using sweeping method in weeds between the lines (30 times/ plot) and the yellow chromotropic traps method (in 3 olive trees per plot). The insects with interest for the study were observed through a stereoscopic microscope and counted, and genitalia were also prepared in order to properly identify the species. A comparation between each capture method and each plot was made. The most of insects were captured in the youngest plot and were found by the sweeping method in weeds between the lines. Insects of *Cicadomorpha* suborder family were found, but has not been identified a significant sample of potential vectors of *X. fastidiosa*. Just one specimen of potential vector was identified from *Aphrophoridae* family – a *Neophilaenus campestris*. Among *Fulgoromorpha*, which are not regarded as potential vectors, specimen of the *Tettigometridae* family were found. Additional studies are required to better control the incidence of these insects in this region and to evaluate different cultivars resistance to *X. fastidiosa*.

Keywords: Morphology; potential vectors; *X. fastidiosa*

Understanding host-plant shifting of *Philaenus spumarius* in UK

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Xylella fastidiosa is a bacterial pathogen responsible for various plant diseases such as Pierce's disease, phony peach disease and citrus variegated chlorosis in the Americas and for Olive quick decline syndrome in Europe. The bacterium is transmitted by xylem feeding insects belonging to the Order *Hemiptera*, sub-order *Auchenorrhyncha*. While the vectors are well understood in the Americas, our knowledge in Europe is less comprehensive. The meadow spittlebug *Philaenus spumarius* was the first vector species identified in Italy (Saponari et al., 2014) with other *Aphrophoridae* such as *Neophilaenus campestris* following (Cavalieri et al. 2019). Host-shifting of *P. spumarius* occurs through-out the season with adults moving from herbaceous to woody hosts in the drier summer months before returning to herbaceous plants for oviposition in the Autumn. The work focussed on understanding if local populations of vectors in the UK behave in a similar fashion. Provisional work was carried out in the summer of 2020 monitoring densities of *P. spumarius* and other *Aphrophoridae* in the understorey and lower canopy of broadleaved woodlands from July to October as well other iconic habitats found in Scotland such as heather heathland. Peak densities of P. spumarius adults were recorded in August: 1.8, 1.1, 0.8, 0.73 and 0.1 insects per m2 (heather heathland, woodland understorey, grassland, field margin and hedgerow respectively). Adult *P. spumarius* abundance in tree canopies was 0.03 insects per m2 compared to 1.1 per m2 in the understorey, whilst *Aphrophora alni* had a peak abundance of 0.69 in canopies compared to 0.06 in the understorey. *Neophilaenus lineatus* was not observed in tree canopies. This early work suggests that *Philaenus spumarius*, although observed in tree canopies, do not migrate in large numbers to canopies as seen in Mediterranean regions. This is not unexpected due to climatic and habitat differences between the two regions. Further work is planned over the next two years to fully understand how insect distribution changes throughout the summer within different habitats including agroecosystems and how this might influence the spread of *Xylella* were it to be introduced.

Philaenus spumarius and *Neophilaenus campestris* as efficient insect vectors for *Xylella fastidiosa* in Majorca (Spain)

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Xylella fastidiosa is a pathogen xylem-limited bacterium detected in the Balearic Islands (Spain) in 2016 and transmitted by xylem-sap feeders insect vectors. The major potential vectors described in the Balearics are the spittlebugs *Philaenus spumarius* and *Neophilaenus campestris* (*Aphrophoridae*). In order to assess the potential transmission efficiency of these vectors, two types of transmission tests were conducted. In the first type, we assessed the natural infectivity of the vectors. For this, *P. spumarius* and *N. campestris* adults were collected from the field and kept in groups of three to five insects in alfalfa (*Medicago* sativa) for 96 hours for the inoculation access period (IAP). The infection status of alfalfa plants was checked at 15, 30, 45 and 60 days after IAP. In the second type of experiment, uninfected adults were caged on *X. fastidiosa* infected grapevine and almond plantsfor 96 hours of acquisition access period (AAP) and then transferred onto healthy. Plants of alfalfa as described for the first type of experiment. In both experiments, the presence of *X. fastidiosa* in plants and insects was established by qPCR analysis. In the first experiment, 21.7% of *P. spumarius* and 15.6% of *N. campestris* (field collected) tested positive for *X. fastidiosa*. About 35% of the plants exposed to *P. spumarius* and 43% of those exposed to *N. campestris* were infected. In the second experiment, 3.8% of *P. spumarius* acquired the bacteria from infected vine plants and 14,3% of the alfalfa plants resulted positive to *X. fastidiosa* after the IAP. In conclusion, both species of spittlebugs found in the Balearics are able to transmit *X. fastidiosa* in laboratory trials. However, its comparative epidemiological role could be different due to host preference, distribution and abundance.

Efficiency of different trap types for the monitoring of *Philaenus spumarius*

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The spittlebug *Philaenus spumarius* (Cicadomorpha: *Aphrophoridae*) is the principal vector of *Xylella fastidiosa* in Europe. Surveys on the occurrence and abundance of this and other spittlebug species are necessary for the identification of endangered crops and natural refugia, risk assessment, the development of prevention strategies, or decision support regarding control measures. Common monitoring techniques for insects comprise active sampling by sweep-netting as well as passive capturing using sticky-traps or pan-traps. The objective of this experiment was to compare the efficiency of the three sampling methods in monitoring of *P. spumarius* in two different habitats with a high density of *P. spumarius*, a vineyard and an extensively managed pasture. The experiment was carried out during July and August 2019. Fifteen trapping stations were randomly distributed in the vineyard while 10 stations were positioned along a transect on the pasture. Each trapping station consisted of a pan-trap, a sticky-trap and an area of 5 m diameter around the traps that was sampled by five sweeps of a sweep-net. Pan-traps were exposed for three, and sticky-traps for seven days. Sweeping was carried out once a week during the replacement of the traps.

Captures of *P. spumarius* could be achieved with all collecting methods. However, the numbers collected by sweep net punctually once a week were significantly higher than those captured by the two continuously exposed trap types. Numbers of captured spittlebugs per week did not differ significantly between sticky-traps and pan-traps. Sweep-netting proved to be the most efficient collection method, both in terms of capture numbers and time spent. If continuous sampling is required, pan-traps and sticky-traps are equally efficient but sticky-traps are by far easier to handle. If the captured insects are intended to be used for subsequent laboratory tests, sweep-netting or pan-trapping are the methods of choice.

Keywords: spittlebugs; survey; vectors

Relative efficacy of different colour sticky traps for the capture of vectors of *Xylella fastidiosa*

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Xylella fastidiosa is one of the main quarantine pathogens of the European Union that is transmitted by xylem-feeder insects (i.e. *Cicadomorpha*). This bacterium affects plant species of great economic importance such as almond, olive, and vine, among others. During 2020 we conducted a trial in Mallorca (Balearic Islands; Spain) to study the efficacy of colour sticky traps to capture spittle bugs (*Aphrophoridae*) which are considered major vectors of *X. fastidiosa in* the EU. Samplings were carried out weekly, from June to December 2020 in three plots: one almond orchard, one olive orchard, and one vineyard. In each plot, 16 traps of each colour (red, yellow, blue and white) were used. Traps were placed using a Latin square design in the tree canopy, cover vegetation and woody bordering shrubs. The in the same plots during the same period of sampling. A total of 89 individuals were captured, 73 *Philaenus spumarius* and 16 *Neophilaenus campestris*, using 3458 sticky traps. The highest number of insects were captured in olive (59.55%), followed by vineyard (22.47%) and almond (17.98%). Depending on the location of the trap, 60.67% of insects were captured in trees, while 20.22% were in the cover vegetation and 19.10% in bordering shrubs. Vectors showed preference for yellow colour (57 captures; $p<0.005$) compared to red, white, or blue colour traps. Sticky traps showed lower number of insects (89 insects) compared to the sampling by using sweeping net (108 insects). Our results highlight that yellow coloured sticky traps are the most effective for capturing vectors of *X. fastidiosa* in the main crops of the Balearic Islands.

Wanted egg parasitoids: *Ooctonus vulgatus* controls *Philaenus spumarius* populations in Corsica and is probably widely distributed in Europe

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Current management of *Xylella fastidiosa* (Xf) epidemics worldwide predominantly involves plant uprooting, soil tillage or insecticide use. Biological control of insect vectors is a promising environmentally friendly way to limit the spread of Xf but this research field has been overlooked until very recently.

Three types of natural enemies of *Philaenus spumarius* have been described: egg parasitoids, larval and adult predators, and adult parasitoids. In the present study, we tried to identify egg parasitoids of *Philaenus spumarius* in Corsica and to give a first assessment of their efficiency in natura. For that purpose, we collected bunches of branches of *Cistus monspeliensis* in winter, checked for the presence of eggs of *P. spumarius* on leaf underside, and monitored insect emergence.

Ooctonus vulgatus Haliday, 1833 was identified by morphological and molecular methods as emerging from 255 *P. spumarius* eggs, out of 808 emerged individuals (total sample size = 1107 eggs). This was the only parasitoid species found and it was recorded on 7 out of 9 sampling sites, with parasitism rates ranging from 4 to 69 %. Species distribution models fitted on all available occurrences of *O. vulgatus* worldwide show that this species is likely to be found commonly in northwestern Europe.

Among known natural enemies of *P. spumarius*, egg parasitoids have a unique combination of features: they kill the pest before it reaches the most harmful stage, and usually exhibit a high level of host specialization. Consequently, we can expect that spatially restricted mass releases in host habitats could decrease Xf transmission without side effects on the local fauna. This probably makes egg parasitoids promising biocontrol agents for the Xf patho-system although level of specialization of the parasitoids and our ability to mass-rear the species that may control all vectors need to be clarified.

Keywords: Pest natural regulation; *Chalcidoidea*; Integrated Pest Management

Xylella fastidiosa vectors show pronounced habitat preferences in Corsican agricultural landscapes

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In Europe, many potential and confirmed vectors of *Xylella fastidiosa* (Xf) are polyphagous but appear to aggregate on their preferred host plants at local scales. For example, *Philaenus spumarius* is found most often on *Asteraceae* on the Californian coast, or on *Apiaceae* and *Rubiaceae* in the meadows of central Italy. However, vectors keep the ability to exploit multiple host-plants, which can be crucial in primary Xf transmission to crops.

In this study, we assessed the habitat preferences of spittlebugs on and in the vicinity of Corsican clementine and olive groves, focusing on four habitats: *Cistus monspeliensis* border, *Dittrichia viscosa* cover, crop foliage and grove ground vegetation. Spittlebug abundance was assessed three times a year during two years on five to nine clementine and olive organic groves. Nymph and adult abundances were quantified based on spittle mass counts and nymph identification in April and sweep net sampling in June and October. Habitat preferences were inferred based on generalized linear mixed models.

Overall, 6647 spittle masses and 1714 adults belonging to four species were found. *Philaenus spumarius* had a significant preference for *Cistus monspeliensis*, and to a lesser extent for *Dittrichia viscosa*. *Neophilaenus campestris* mostly occurred on ground vegetation of the grove and on *Dittrichia viscosa*. *Lepyronia coleoptrata* was less abundant on crop foliage than on ground vegetation and *Aphrophora alni* showed an even abundance in all habitats.

This study demonstrates the specialization of *P. spumarius* on *C. monspeliensis* in the diversified Corsican landscape as well as strong habitat preferences for *N. campestris*. All recorded species were able to colonize clementine or olive foliage, but rarely, and in similar abundancies for all species. Should they be proven equivalent effective vectors, this suggests similar roles of these four species in Xf transmission to crops.

Keywords: Insect community, plant-insect network, *Auchenorrhyncha*

Attractiveness of different colored sticky traps for spittlebug vectors of *Xylella fastidiosa*

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Philaenus spumarius is the main vectors of *Xylella fastidiosa* in Europe. For integrated pest management, the monitoring of the pest is crucial to planning an efficient and sustainable control strategy. The monitoring of spittlebug adults is carried out mainly using the sweeping net. Nevertheless, several studies have reported its ineffectiveness in sampling insects from the olive canopy. Moreover, using the sweeping net requires quite a lot of time and a skilled operator. So far, only one study has been carried out on the attractiveness of different color sticky traps for adults of P. spumarius. The results showed that the most attractive one was yellow. Nevertheless, the use of yellow sticky traps in a field has highlighted a low efficacy in monitoring spittlebugs. In order to improve the knowledge on this topic a new studies on the attractiveness of different colors are needed. Within the Life Resilience project, a field trial, aimed at testing different color sticky traps, has been carried out in three different olive groves in Tuscany in the 2020 fall. The tested colors were white, red, blue, brown, dark green, yellow (as positive control) and transparent as negative control. For each color, two 20x25 cm double-sided sticky boards were positioned in the olive grove respectively at 50 and 100 cm from the ground. Traps were controlled every fifteen days and trapped specimens of *P. spumarius* and *N. campestris* were removed, identified, sexed and counted. Moreover, during every on-site visit a monitoring of the population density was carried out by means of the sweeping net. As previously recorded, yellow colored sticky traps showed the greatest attractiveness towards *P. spumarius*, especially males, while only a few *N. campestris* were caught by the traps overall. Further studies on the response to colors are planned in order to clarify these preliminary results.

Response of *Philaenus spumarius* and *Neophilaenus campestris* to potential semiochemicals

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Before the *Xylella fastidiosa* outbreak in Europe and the identification of spittlebugs as the main vectors of this xylem-inhabiting bacterium, little was known about this insect group. The need of effective control tools has highlighted the lack of knowledge on several aspects of spittlebugs' ecology, such as their response to olfactory stimuli and the potential role of different volatile compounds as semiochemicals. Recently, the antennal sensilla fine structure and the response to several volatile organic compounds were investigated in Philaenus spumarius. Although its antennal chemoreceptors are limited, the meadow spittlebug seems to be able to recognize and react to some substances. While the study of P. spumarius chemical ecology is ongoing, for *Neophilaenus campestris* this topic is still uninvestigated. In order to evaluate the response of P. spumarius and N. campestris adults to volatile organic compounds, several Y-tube olfactometer bioassays were carried out. Bergamot essential oil, farnesene, nonanal and undecenal were selected through a preliminary electroantennographic examination. In addition to these compounds, insect body extracts obtained from 5 males and five females of both species were tested too. The average linear speed of the insect in the olfactometer and time spent in the two arms (treatment vs. control) were considered. In response to almost all the substances, a decrease in the linear speed for both males and females of N. campestris was evidenced in comparison to pure air. Males of both species responded to farnesene remaining for a longer time in the arm containing the substance compared to the arm with pure air. Moreover, when considering the body extracts of males or females both species showed a linear speed significantly different compared to the control tests. These results may suggest that one or more volatile compounds extracted from spittlebug bodies could play a role as semiochemicals, especially in *N. campestris*.

Detection of Philaenus (Hemiptera: Aphrophoridae) DNA in the gut of spiders, using PCR-based gut-content analysis

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With the fast spread of *Xylella fastidiosa* throughout Europe, it is important to identify natural enemies of *Philaenus spumarius* (Hemiptera: *Aphrophoridae*), which is the main vector of this pathogen. Generalist predators, such as spiders, can play a key role in the control of *P. spumarius*. However, the determination of predation by spiders in the field is challenging. The use of PCR-based techniques may be a reliable approach to accurately detect these predation events. Here, a new set of 20 primers were designed and tested to detect the presence of *Philaenus* sp. DNA in the gut of spiders. Primers' specificity was tested with species closely related and not related to *P. spumarius* and primer sensitivity was evaluated by using different concentrations of *P. spumarius* DNA. To estimate the digestion time at which prey DNA is detectable within the predator after ingestion, feeding trials with the spider *Xysticus acerbus* Thorell, 1872 (*Thomisidae*) were performed. One primer pair showed great sensitivity and specificity for *Philaenus* and when applied in the feeding assay the primer pair successfully amplified the degraded DNA of *Philaenus* in the gut of *X. acerbus*. According to the Probit regression, there is a 50% probability of detecting DNA from *Philaenus* 82 hours after ingestion. The practical applicability of this primer pair was validated by confirming, by DNA sequencing, the presence of consumed *Philaenus spumarius* within spiders collected in field. This study demonstrates that this molecular-based diagnostic tool can provide an estimation of predator efficiency of spiders over *Philaenus* spp. in the field.

Keywords: *Philaenus*-specific primers; *Xylella fastidiosa*; biological control

Olfactory behavior of *Philaenus spumarius* (Hemiptera: Aphrophoridae) to two naturally occurring volatile compounds on almond, olive, and vine leaves

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Olive groves, almond orchards, and vineyards are characteristic elements of the landscape in the Mediterranean basin. Currently, these crops are threatened by the vector-borne plant pathogen *Xylella fastidiosa*, which is rapidly transmitted and spread by insect vectors, such as *Philaenus spumarius*, the main vector in Europe. Understanding the olfactory response of *P. spumarius* is of outmost importance since the response to chemical signals in the environment can affect insect survival and reproduction, eliciting attractive or aversive behavior. In this work, we aimed at evaluating the olfactory response of adults of *P. spumarius* to two volatile compounds common in almond, olive, and vine leaves. For this, the volatile profile of each crop was assessed by headspace solid-phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC/MS). The volatile compounds cis-3hexenyl acetate and cis-3-hexen-1-ol are present in all the crops. The behavioral responses to the volatile compounds were assessed by recording individually 30 Individuals of both sexes of *P. spumarius* for 20 min in a four-arm olfactometer. The volatile compounds were tested at 5, 10, 20, 30 µg/µL and a stream of purified air and sunflower oil were used as controls. At the lowest concentration, females and males were significantly attracted by the two volatile compounds ($P<0.01$). Moreover, they showed a higher frequency of visits and a longer permanence in the areas with cis-3hexenyl acetate and cis-3-hexen-1-ol. However, when the individuals were exposed to the volatile compounds at 10 µg/µL, females chose more often the stream of purified air ($P<0.01$), whereas the males presented no significantly differences in their choice ($P= 0.093$). At the highest concentrations, no significantly differences were detected among treatments. Low concentrations of these volatiles may attract *P. spumarius*, however, higher concentrations can saturate the olfactory sensilla affecting the choice.

Keywords: four-choice olfactometer; plan pathogen; volatile profile

A web-interface database for the identification of vectors of *Xylella fastidiosa* in Europe

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Identification of adult vectors of *Xylella fastidiosa* (Xf) based on morphological characters can be difficult for non-specialists. The identification of eggs or larvae is even more difficult and sometimes impossible. To circumvent these difficulties and enable large survey of populations of insect vectors in Europe we have developed a web-interface database of COI barcodes. COI barcodes were generated with a high-throughput approach involving two PCR steps to target COI and index samples followed by Illumina sequencing. Multiple quality controls were implemented including i) non-destructive DNA extraction that allows vouchering and morphological re-examination by taxonomists; ii) bioinformatic controls to discards possible contaminants, PCR or sequencing errors, NUMTs and heteroplasms.All barcodes have been uploaded in a web-interface database (Arthemis DB@se: http://arthemisdb.supagro.inra.fr/DefaultInfo.aspx?Page=Home). Metadata associated with all records are available online, as well as biological data on vectors and pictures. Users can identify a query sequence through BLAST comparison against our reference library. Currently, the database hosts 636 barcodes for 72 species of potential vectors of Xf: 490 sequences representing 43 European species and 145 sequences representing 29 non-European species. All frequent species have been barcoded. Missing species are rare or localized and do not represent an important threat for European agriculture.COI allows reliable identification for 80% of the species, but we observed issues for the remaining 20%. The most important issue concerns Philaenus spumarius and its close relative P. tesselatus. These two species are hardly distinguishable even with male genitalia and share the same COI sequence. It was also difficult to cross validate morphological and molecular results for some species within the genera *Neophilaenus* and *Aphrophora.*

Keywords : Xf vectors, barcoding, identification

Presence, diversity and seasonal fluctuation of *Xylella fastidiosa* potential vectors and other Auchenorrhyncha (Hemiptera) in olive agroecosystems with different management systems

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Presence, seasonal abundance and diversity of *Xylella fastidiosa* potential vectors species and other Auchenorrhyncha were studied in olive orchards under different management systems. Auchenorrhyncha were monitored monthly in 6 olive groves at Istiea (Central Greece) and Chania (South Greece) for 13 months using Malaise traps and sweeping-net, as a sampling strategy to collect even the most, or the least-mobile Auchenorrhyncha within the orchards. The most abundant *X. fastidiosa* potential vector was *Philaenus spumarius* , followed by *Neophilaenus campestris*, *Cercopis sanguinolenta*, *Lepyronia coleoptrata* and *Neophilaenus lineatus*. Olive orchards in Chania had a lower abundance and diversity in X. fastidiosa potential vectors, as well as in total Auchenorrhyncha. The abandoned olive orchard hosted the highest population of potential vectors in Chania, but the lowest in Istiea, compared with the respective cultivated one. The organic farms were more diverse in Auchenorrhyncha compared to the other management systems in both regions. Weed control provided the most probable explanation for the significant Auchenorrhyncha population fluctuation among olive orchards. However, insecticide applications against olive fruit fly did not affect Auchenorrhyncha abundance. The conventional orchard in Istiea displayed the highest abundance and species richness of the potential vectors of X. fastidiosa, mainly during autumn, when dried weeds remained in the orchard after herbicide application. Regarding monitoring methods, sweeping-net was the method with the most abundant potential vector collection in general, while Malaise traps were more efficient for collecting potential vectors during migration to the dried weeds (in fall).

Keywords: olive quick decline syndrome (OQDS); potential vector; spittlebugs; Aphrophoridae; conventional; organic; abandoned

POSTER SESSION:
Surveillance and early detection tools

Title	Presenter	DOI
Testing and validation of methods to diagnose the causative agent of Pierce's disease in different kinds of substrate	**Prikhodko S,** All-Russian Plant Quarantine Center" (FGBU "VNIIKR") (RU)	**https://doi.org/10.5281/zenodo.4682098**
An improved delimiting survey approach for *Xylella fastidiosa*	**Lázaro E,** Institut Valencià d'Investigacions Agràries, Moncada (ES)	**https://doi.org/10.5281/zenodo.4682103**
Measuring the threat from a distance: a sentinel plantation in Palma de Mallorca to test the susceptibility of Belgian trees to several subspecies of *Xylella fastidiosa*	**Casarin N**, UCLouvain (BE)	**https://doi.org/10.5281/zenodo.4682118**
Salicaceae, a potential plant network for the spread of *Xylella fastidiosa* in temperate regions	**Casarin N**, UCLouvain (BE)	**https://doi.org/10.5281/zenodo.4682127**
Sequence-typing of *Xylella fastidiosa*: new perspective for MLST analysis by Nanopore sequencing	**Nicoloso VM,** Institute for Sustainable Plant Protection, CNR, Bari (IT)	**https://doi.org/10.5281/zenodo.4682131**
EFSA pest survey card on *Xylella fastidiosa*: how to define the detection method?	**Mattion G,** Animal and Plant Health Unit, European Food Safety Authority (EFSA), Parma (IT)	**https://doi.org/10.5281/zenodo.4682148**
EFSA pest survey card on *Xylella fastidiosa*: how to characterise the host plant population?	**Mattion G**, Animal and Plant Health Unit, European Food Safety Authority (EFSA), Parma (IT)	**https://doi.org/10.5281/zenodo.4682159**
Integrated IoT monitoring system and data science platform to monitor plant conditions under biotic and abiotic factors	**Santini M,** Fondazione Centro Euro-Mediterraneo sui Cambiamenti Climatici (CMCC), Viterbo (IT)	**https://doi.org/10.5281/zenodo.4682166**
FREE@POC: Towards an instrument-FREE future of molecular diagnostics at the Point-Of-Care	**Minafra A,** Institute for Sustainable Plant Protection, CNR, Bari (IT)	**https://doi.org/10.5281/zenodo.4682774**
Results of official annual survey for *Xylella fastidiosa* in Montenegro	**Popović T,** Administration of Food Safety, Veterinary and Phytosanitary Affairs, Podgorica (ME)	**https://doi.org/10.5281/zenodo.4682786**
Sentinel insects to anticipate, detect and monitor *Xylella fastidiosa* outbreaks	**Farigoule P,** AgroParisTech, Paris, France & CBGP, INRAE, CIRAD, IRD, Montpellier SupAgro, Univ. Montpellier, Montferrier-sur-Lez (FR)	**https://doi.org/10.5281/zenodo.4682793**

Xylella fastidiosa is not detected yet in Jordan: survey results	**Al-Karablieh N**, Department of Plant Protection, School of Agriculture, The University of Jordan, Amman (JO)	**https://doi.org/10.5281/zenodo.4682803**
Optimizing molecular assays to support early detection of *Xylella fastidiosa* in host plants	**Reppa CI,** Benaki Phytopathological Institute, Kifissia (GR)	**https://doi.org/10.5281/zenodo.4682820**
Setting up of efficient sampling schemes to detect *Xylella fastidiosa* in Apulian olive groves	**Santoro F,** Mediterranean Agronomic Institute of Bari (IT)	**https://doi.org/10.5281/zenodo.4682832**
Phenometabolomics of olive quick decline syndrome using nuclear magnetic resonance, high resolution mass spectrometry, hyperspectral reflectance, and integrative chemometrics analysis	**Ahmed EMFMH,** CIHEAM – Mediterranean Agronomic Institute of Bari, Valenzano, and Department of Civil, Environmental, Land, Building Engineering and Chemistry (DICATECh), Polytechnic University of Bari (IT)	**https://doi.org/10.5281/zenodo.4682845**
Use of indicator plants for the early detection of *X. fastidiosa*	**Bergsma-Vlami M**, Dutch National Plant Protection Organization (NPPO-NL) (NL)	**https://doi.org/10.5281/zenodo.4682921**
Diagnostic pipeline and large-scale monitoring: the experience in the Apulian outbreak	**Saponari M,** Institute for Sustainable Plant Protection, CNR, Bari (IT)	**https://doi.org/10.5281/zenodo.4682925**
Capturing efficacy assessment of antibodies against *Xylella fastidiosa* through Surface Plasmon Resonance	**Sarcina L,** Università degli Studi di Bari Aldo Moro, Bari (IT)	**https://doi.org/10.5281/zenodo.4682954**
Xylella fastidiosa field detection combining Loop-Mediated Isothermal Amplification (LAMP) with Long-read Nanopore sequencing	**Mota M,** Faculdade de Ciências, Universidade da Beira Interior (UBI), Covilhã, and Centro de Apoio Tecnológico Agroalimentar (CATAA), Castelo Branco (PT)	**https://doi.org/10.5281/zenodo.4682970**
Enhancing the UK diagnostic capabilities for *Xylella fastidiosa*	**Walshaw J**, Fera Science Ldt, York (UK)	**https://doi.org/10.5281/zenodo.4682982**
Xylella fastidiosa: Imminent Risk to Food Security in Near East and North Africa Region	**Yaseen T,** Food and Agriculture Organization of the United Nations (FAO)	**https://doi.org/10.5281/zenodo.4714490**

Testing and validation of methods to diagnose the causative agent of Pierce's disease in different kinds of substrate

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A number of host plants of *Xylella fastidiosa* (Xf) are imported to the Russian Federation such as *Vitis* spp., *Prunus* spp., *Vaccinium* spp., *Olea* sp, as well as ornamental plants. The main difficulty for the diagnosis of Xf in imported plants for planting is the need to isolate the bacterium from matrices such as wood and roots. Samples (seedlings and cuttings) to be tested are in a dormant state. The VNIIKR bacteriology laboratory optimized the preparation of the plant extract by including a purification and a concentration step. To extract nucleic acids from this extract, the PhytoSorb kit (Syntol, Russia) is used. This DNA extraction is based on the sorption of DNA on silica gel-coated magnetic particles followed by sedimentation with a precipitating reagent. For the detection of Xf DNA in the sample, the real-time PCR according to Harper et al., 2010 shows the best results. This test is routinely used in the bacteriology laboratory as a screening test. The validation of sample preparation, as well as diagnostics of Xf was carried out in the Republic of Crimea, where approximately 250 samples of Xf host plants were analyzed. At least 30 indigenous species of cicadas (Hemiptera, Auchenorrhyncha) are present in the territory of the Republic of Crimea and the meadow spittlebug, *Philaenus spumarius L.*, is common in various plant communities. To extract nucleic acids from insects, the DNA-Extran-2 kit (Syntol, Russia) is routinely used. In this case, the liquid-phase method of genomic DNA extraction is applied, which can also be used for bacterial DNA extraction and has shown good results when working with Xf-infected material.

An improved delimiting survey approach for *Xylella fastidiosa*

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The EU plant health legislation enforces the implementation of intensive surveillance programs for *Xylella fastidiosa* (Xf) as a quarantine pathogen (Commission Implementing Regulation (EU) 2020/1201). After a Xf outbreak, delimiting surveys must be implemented to delineate the extent of the pathogen and to execute disease control. The surveillance efficacy can be enhanced by increasing inspection and sampling intensities. Budget constraints often limit survey efforts, thus making it necessary the optimization of surveillance strategies. A sequential adaptive delimiting survey involving a three-phase and a two-phase design with increasing spatial resolution was simulated for the Xf demarcated area in Alicante, Spain. Based on the official survey data of 2018, inspection and sampling intensities were estimated using an optimization algorithm specified under the sequential adaptive delimiting strategy. The sampling intensity thresholds estimated were evaluated by quantifying their effect on the estimation of Xf incidence. This strategy made it possible to sequence inspection and sampling considering increasing spatial resolutions, and to adapt the inspection and sampling intensities according to the information obtained in the previous, coarser, spatial resolution. Our results show that sequencing and adapting inspection and sampling to increasing spatial resolutions allows accurate delimitation of the infested zone while reducing the overall survey efforts, thus improving the efficiency of the surveillance program. From a methodological perspective, our approach provides new insights into alternative delimiting designs and new reference sampling intensity values.

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Keywords: Adaptive sampling; Bayesian spatial statistics; Survey design

Measuring the threat from a distance: a sentinel plantation in Palma de Mallorca to test the susceptibility of Belgian trees to several subspecies of *Xylella fastidiosa*

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Sentinel plantations are a promising tool to collect valuable information on plant-pathogen interactions. While it has proven its efficiency to discover new significant threatening organisms (Kenis et al., 2018; Vettraino et al., 2017), this technique has also the potential to identify new host plants for known pathogens. The objective of this study was to investigate the susceptibility of selected Belgian potential host plant species to *Xylella fastidiosa* by establishing and monitoring ex-patria plantings (EPPO 2020) in a *Xylella*-contaminated area, the campus of the University of the Balearic Islands (UIB) situated in Palma de Mallorca. First, official approval was obtained from the local government and from the UIB Authorities. Then, selected plant species (*Prunus domestica* cv. opal, *Salix alba*, *Quercus petraea*), were acquired from Belgian producers. Although they were provided with phytosanitary certificates, they were also checked for *X. fastidiosa* before 27 plants of each species were brought to Spain and planted in March 2018. Later on, they were regularly submitted to regular symptom observations and molecular analyses. Simultaneously, within a 100 meters radius area around the plantation, the abundance and the infectivity of the potential insect vectors, as well as the sanitary state of the local vegetation, were monitored. The survey is still ongoing. So far, no infection by *X. fastidiosa* has been observed, possibly due to the young age of the trees and the recent establishment of the assay, as well as to the scarcity of the ground herbaceous vegetation and vectors during the first year. This study underlines the hurdles that may be encountered with sentinel plants. Yet it provides a methodology highlighting key features to address *X. fastidiosa* studies through sentinel plantations.

Reference:

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Salicaceae, a potential plant network for the spread of *Xylella fastidiosa* in temperate regions

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Xylella fastidiosa represents a definite threat for Mediterranean flora and southern Europe, as shown by the different outbreaks in France, Italy, Portugal, and Spain. The threat for northern temperate areas is considered more limited based on climate-suitability modelling, but should not be underestimated considering *X. fastidiosa* in America. The risk for these regions depends mostly on the presence of efficient host plants and insect vectors combinations. Considering this and the scarcity of data about the susceptibility of indigenous European potential host plants, several candidate host plants for Belgium have been evaluated. After a pre-screening by mechanical inoculation, we focused on two Salicaceae, *Populus tremula* and *Salix alba*. The family Salicaceae is been associated with several potential insect vectors. It is characteristic of the riparian areas, which have been pointed out to be a favorable niche for both *X. fastidiosa* and insect vectors. Furthermore, Salicaceae species are also distributed in forests, along the roads and the hydrographic network, therefore having the potential to act as steppingstones in the potential establishment and spread of the pathogen. Our study is carried out by monitoring the bacterial spread and multiplication in the plants after mechanical inoculations under controlled biosafety conditions. The GFP-labeled strain KLN59.3 is used to perform confocal microscopy and scanning electron microscopy to understand the distribution of bacteria in xylem vessels, along with real-time quantitative PCR and re-isolation of the bacteria with the aims to fulfill Koch's postulates. The research aims at providing scenarios to anticipate the occurrence of such a high-risk pathogen as *X. fastidiosa* in EU temperate regions, so that appropriate risk reduction options can be rapidly deployed in case of detection in these environments.

Sequence-typing of *Xylella fastidiosa*: new perspective for MLST analysis by Nanopore sequencing

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Multi-locus sequence typing (MLST) is a widely used methodology for typing strains of *Xylella fastidiosa* (Xf) (Yuan et al., 2010; EPPO 7/24(4)). This approach is based on the sequence analyses of 7 housekeeping genes, on which the allelic profile and the sequence type (ST) is determined. Given the difficulties to isolate and culture Xf, the MLST analysis is often performed on total plant DNA instead of using bacterial DNA recovered from cultured isolates. In some cases, this may result in weak amplifications or PCR failures. In order to overcome this issue and speed up the sequencing step we tested the Oxford nanopore technology as an alternative approach to Sanger sequencing. To this end, we used the Oxford nanopore MinION device for sequencing the PCR products obtained from 4 olives and from 3 samples of *Dodonaea purpurea*. Previous analyses had identified in these infected sources only alleles corresponding to the ST53 genotype. PCR barcoding Nanopore kit SQK PBK004 was used to sequence the 7 amplicons. Reads having passed the EPI2ME quality filter were launched in the MLST 2.0 pipeline (Larsen et al., 2012). Results showed that in all samples, the expected allele for each PCR product was obtained, i.e. confirming the occurrence of the ST53 genotype in all tested samples. Indeed, for some genes multiple alleles were identified. However, the occurrence of these additional alleles was not confirmed when the corresponding PCR products were cloned and a large number of clones sequenced. Overall, although this technology suffers from high error rates, its use for MLST analysis proved to be cost effective and time saving, allowing to gather a rapid snapshot of the Xf genotype associated to a given host plant/outbreak.

EFSA pest survey card on *Xylella fastidiosa*: how to define the detection method?

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EFSA developed the "Pest survey card on *Xylella fastidiosa*" for the preparation of surveys, with the aim of guiding the Member States in gathering information needed for designing statistically sound and risk-based surveys for the pathogen. In particular, it addresses the key question on how to detect the organism. The method of detection needs to be defined and consists of a sequence of operations starting from the selection of the units to inspect, the inspection itself, the sampling of plant material and finally the diagnostic and testing in the laboratory using available protocols. The inspection unit is the elementary subdivision of the host plant population targeted by the survey and to which the detection method is applied. For X. fastidiosa, the inspection units are the individual host plants. A major challenge for the detection of the bacterium is that both symptomatic and asymptomatic plant material should be scrutinised and systematically sampled. This is because infected plants can be asymptomatic for a long and variable period, and symptoms can be confused with drought-induced stress and other biotic or abiotic stresses. Nonetheless, an accurate visual examination to detect *Xylella*-like symptoms plays a major role during the collection of samples. The performance of the detection method, defined as the overall method sensitivity, should be estimated based on the available information. It is suggested to combine the sampling effectiveness, related to the sampling procedures, and the diagnostic sensitivity, defined by the laboratory tests. Characterising the detection method and its overall sensitivity is necessary for designing a robust survey, and to calculate the survey effort required to detect the pest in terms of number of inspections, samples, and laboratory tests.

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EFSA pest survey card on *Xylella fastidiosa*: how to characterise the host plant population?

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EFSA developed the "Pest survey card on *Xylella fastidiosa*" for the preparation of surveys with the aim of guiding the Member States in gathering information needed for designing statistically sound and risk-based surveys for this pest. Characterising the host plant population targeted by surveys provides an answer to where to perform survey activities. This is a major challenge for surveillance of *X. fastidiosa* as it can be hosted by about 600 different plant species, growing throughout the EU territory in a variety of environments. For assisting surveyors in their preparatory tasks, the target population can be structured and subdivided in epidemiological units, in which the probability of infection by the bacterium is homogeneous. For this purpose, the "Host plant selection tool" built within the pest survey card provides guidance on the selection of host plants, allocating them in four categories of land use: agricultural, urban, forest and other areas. Rationales are also provided with the purpose of meeting the objectives of the surveys, such as the susceptibility of infection by multiple *X. fastidiosa* subspecies, the susceptibility of feeding by insect vectors, and the expression of symptoms, since some host species undergo an asymptomatic period. In addition, to prepare a risk-based survey, the target population can be subdivided based on the risk of introduction of *X. fastidiosa* in an area or on the risk of infection of some host plant species. The risk factors can be introduced in the survey design to better target the survey efforts. An accurate characterisation of the target population using the proposed methodology will help surveyors to design robust statistically sound and risk-based surveys for *X. fastidiosa*.

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Integrated IoT monitoring system and data science platform to monitor plant conditions under biotic and abiotic factors

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In the context of XF-ACTORS project, a IoT based monitoring system has been established and tested in the field - for an olive grove in Puglia (Italy) affected by Xf - to measure in near real-time some parameters proxies of trees' conditions and vulnerability under both abiotic (climate) and biotic factors. The system is based on the TreeTalker (TT) technology, comprising multiparametric sensors to monitor water transport in trees, trunk humidity and diametrical oscillations, spectral characteristics of the leaves and microclimatic parameters (temperature, relative humidity). In particular, the sap flow density can be retrieved according to the Heat Balance Method (Granier 1985) after measuring the temperature of two 20 mm long probes inserted into the stem wood at 10 cm distance along the trunk vertical axis; the probe in the higher position is heated while the lower one provides the reference temperature.The TT system collects and transmits data at hourly time frequency, thanks to a LoRa based wireless connection, to a node managed by another microcontroller (TT-Cloud) serving a few tens of devices in a cluster. The TT-Cloud is in turn connected to the internet via the GPRS network and sends data to a computer server. Here, raw data are subject to ETL procedure that allows data Extraction from the TT-Cloud source, data Transformation by cleaning and converting them into variables with eco-physiological meaning, and finally data Loading to insert them into the target spatio-temporal database, adopting proper storage format/structure for querying and analysis purpose. From here, data can be further elaborated and visualized, e.g. into useful statistics, through a tailored Data Science environment.The preliminary results on sap flow density are here presented as they can give important information about the impact of Xf that is known obstructing xylem vessels, reducing hydraulic conductivity and thus affecting evapotranspiration.

FREE@POC: Towards an instrument-FREE future of molecular diagnostics at the Point-Of-Care

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The main purpose of Point-of-Care (POC) testing is to decrease the time between specimen collection and detection results, leading to faster intervention and treatment and reduced costs, avoiding the need of large centralized laboratories.The project FREE@POC, started in January 2020 and funded by the H2020-Future and Emerging Technologies OPEN Program, aims to develop an instrument-free molecular diagnostic platform for infectious diseases-testing (HIV, Influenza and Covid-19) in human samples and pathogens (*Xylella fastidiosa*) in plant tissue. The Project is coordinated by Foundation for Research & Technology – Hellas (FORTH) (Greece) and involves other 5 partners: Institute for Sustainable Plant Protection (Italy), University College London Hospital (UK), GNOSIS (Greece), Institute Pasteur (France), Africa Health Research Institute (South Africa).The overall purpose of the project is to employ a new class of molecular tools (like LAMP) for efficient and specific amplification of target nucleic acids at room temperature (RT) and in crude samples, inside disposable reaction units. This instrument-free molecular diagnostic approach will be fully quantitative thanks to a novel digital colorimetric detection method using a smartphone to capture and analyze the output. Rapid transmission of test results to the patients' file, doctor's office, public health officials or a phytosanitary surveillance center will also be implemented.The end-users of the POC tests validated in the project will be healthcare workers at the hospital, intensive care unit or peripheral lab; care providers/nurses at a health post or community center; patients at home or in resource limited areas; farmers, nurserymen or plant inspection service at the field or entry-points.

Keywords: molecular diagnostics, LAMP, human pathogens, *Xylella*

Results of official annual survey for *Xylella fastidiosa* in Montenegro

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Xylella fastidiosa (Xf) is one of the most important bacterial pathogens worldwide causing several diseases in more than 350 cultivated and wild plant species. The bacterium is listed as a quarantine organism in the EU as well as in Montenegro. In order to detect potentially infected material, surveys were carried out since 2015. During 2019, survey of Xf was conducted in places with an increased phytosanitary risk, such as the Montenegrin coast, including areas near the port of Bar and along main roads, plant production sites and urban areas, covering especially the highest risk host plants (olive trees and ornamentals plants in nurseries). The potential host plants of Xf were inspected and sampled during the period of their active growth. A total of 40 samples of suspected plant material (*Olea europaea, Myrtus communis, Nerium oleander, Lavandula spp., Rosmarinus officinalis, Laurus nobilis*) were collected. Symptoms on these plants varied and included necrosis and wilting of leaves, marginal leaf chlorosis and defoliation, twigs dieback. Since Xf is a vector-transmitted bacterium, five adults of *Philaenus spumarius*, were collected from two sites in Montenegro, and tested for the presence of Xf. Real-time loop-mediated isothermal amplification (real-time LAMP) (Harper et al., 2010; Yaseen et al., 2015) was applied for the detection of Xf in the samples of plant and insect material. LAMP system consisted of a portable ICGENE mini device (Enbiotech S.r.L., Italy) and a ready to use diagnostic kit "*Xylella* Screen Glow". During the analysis, only the positive control developed a distinctive amplification curve. No positive samples were detected. The advantages of real-time LAMP method are easy handling and rapid results of the analysis. Generally this test is suitable for the detection of Xf in plant and insect vector material. Results of the 2019 survey and LAMP laboratory analyses confirmed that Xf was not present in Montenegro.

Keywords: *Xylella fastidiosa*, survey, LAMP

Sentinel insects to anticipate, detect and monitor *Xylella fastidiosa* outbreaks

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Monitoring of plant pathogens is usually carried out by analyzing symptomatic plant material. However when plants are frequently asymptomatic, which is the case for *Xylella fastidiosa* (Xf), this approach is problematic because it limits our ability to early detect the bacterium hence to understand and control its spread. To complement plant survey and facilitate large-scale monitoring, we propose to generalize the use of insect vectors as sentinels. We tested this approach in Corsica using *Philaenus spumarius*, an ubiquitous insect vector, as sentinel. We will present a high throughput method based on a two-step PCR approach followed by Illumina sequencing. Two genes of the MLST of Xf were targeted to identify the three main subspecies of Xf (multiplex, pauca or fastidiosa). This approach enables larger screening than classic (nested) PCR approaches followed by Sanger sequencing. We used this approach to monitor the spatio-temporal prevalence of Xf within vector populations (27 populations of 30 specimens). Our results show that Xf occurs throughout Corsica with a prevalence in insects up to 43% depending on the region and the year of sampling. Moreover, we show that Xf is present in ecosystems assumed uncontaminated on the basis of plant monitoring. No spatial, temporal or vegetation-related pattern of Xf prevalence in insects seem to emerge from our first data. Our results confirm that Xf introduction in Corsica likely predates 2015, it suggests ecological resilience of ecosystems and argues in favor of using insect vectors for Xf survey at large scale in Europe.

Xylella fastidiosa is not detected yet in Jordan: survey results

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The bacterium *Xylella fastidiosa* is a destructive pathogen which attacks a wide range of hosts of more than 350 plant, causing various diseases. Recent outbreak of X. fastidiosa in olives and other major crops in many countries in the European Union and other countries in the region is considered a serious threat to the Jordanian agricultural sector. Therefore, a survey was conducted during the years 2016-till now for the presence of this bacterium in Jordan on different hosts including olive (no. of samples: 975), grapevine (560), stone fruits (670), pome fruits (80), citrus fruits (790) oleander (120), rosemary (225) ornamentals plants (985) grown all over Jordan. Samples were taken from plants showing suspicious symptoms and from asymptomatic plants, and potential Hemiptera insect vector samples were also collected. All plant samples were tested for the presence of *X. fastidiosa* using commercial ELISA kits, followed by molecular confirmation by conventional PCR using 3 set of primers. Insect's samples were tested using real a time LAMP PCR commercial kit. All tested samples did not show any positive results in comparison to the positive controls. These results demonstrate that X. fastidiosa is not detected yet in Jordan. However, extensive monitoring program and checking the borders are needed to prevent the entry of this pest into the country.

Keywords: *X. fastidiosa*, ELISA, Jordan

Optimizing molecular assays to support early detection of *Xylella fastidiosa* in host plants

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Efficient sampling approaches combined with reliable, fast testing procedures are crucial factors in surveillance for early detection of *Xylella fastidiosa* (Xf). In the frame of the European project 'XF-ACTORS', work was performed on developing effective sampling methods to support reliable detection of Xf, particularly in asymptomatic plants. The objectives of the work were to: (a) evaluate the efficiency of two methods for DNA extraction from plant material (1 g), employing two commercial kits, one silica-column-based and one magnetic-bead-based, in automated DNA-extraction devices. (b) test the performance of both DNA extraction methods in pooled (10 g) plant samples. c) evaluate the sensitivity of an isothermal DNA amplification and detection system applied on crude plant material preparation without DNA purification, using a portable fluorometer (AmpliFire, Douglas Scientific). The plant material was derived from cultivated host species of high economic importance and wide distribution in Greece: *Olea europaea*, *Prunus cerasifera*, *Ficus carica*, and various ornamentals with a major role in Xf epidemiology: *Lavandula* sp., *Nerium* sp., *Pelargonium* sp., *Pistacia* sp., *Juniperus* sp. and *Tagetes* sp. It was spiked with different levels of inactivated Xf cells. The DNA extracts were assessed for their quality (A260/A280), quantity (ng/μl) and performance in qPCR protocols recommended in EPPO/ISPM diagnostic standards. In the isothermal DNA amplification and detection system experiment, the plant material was derived from: *Polygala myrtifolia*, a species used in this project as an indicator host for monitoring the presence of Xf insect-vectors, and *Myrtus microphylla*, a species exhibiting strong inhibition of amplification in conventional PCR. The results obtained contribute to the improvement of sampling and molecular testing of plants in Xf surveillance programs, supporting reliable early detection of this pathogen, as far as the effectiveness of the selected DNA extraction method is concerned, especially when pooled samples are tested.

Keywords: detection; qPCR; pooled samples

Setting up of efficient sampling schemes to detect *Xylella fastidiosa* in Apulian olive groves

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In the absence of an accurate sampling scheme for monitoring *Xylella fastidiosa* in Apulian olive groves, a "parcel scale" (single olive grove) sampling approach was developed in this study based on official 2016-2019 monitoring data. A total of 34 olive groves of different shapes were extracted within 13 selected outbreak areas with true incidence ranging from 1.96% to 38.29%. A SADIE analysis revealed for most outbreaks an aggregate spatial distribution of infection. Three types of sampling schemes were chosen and applied to the selected olive groves: Random (RS), Systematic (""Simple"" SS and ""in Groups"" GS) and Perimetric (PS). For SS and GS, different inter-plant distances (10, 25, 35, 50, 70, 90, 120 and 150 meters) were evaluated and compared with the corresponding RS scheme. The process of local spread of infection within the olive groves was established using the ""Random walks"" mechanism: generating 100 scenarios of infection patterns for each olive grove, ranging between 0 and 50 % of the total number of trees. In simulations conducted in MATLAB between the different sampling schemes, total errors (RMSE) and introduced biases (MBE) were evaluated and compared.

The SS, GS, and RS schemes introduced increasing RMSE values from 1.72 to 81.295 as a function of the percentage of trees sampled (from 83% to 0.65% of total trees at inter-distances between 10 and 150 meters). RS schemes performed best at 10-meter inter-distances, while SS schemes for inter-distances between 35 and 70 meters. The SS scheme at 35 meters was the best for 68.75% of the analysed olive groves, introducing a low MBE. The PS scheme (corresponding to two rows of trees along the perimeter of the grove) detected the presence of at least one positive tree in all olive groves studied.

Keywords: SADIE, Infection patterns, Random walks

Phenometabolomics of olive quick decline syndrome using nuclear magnetic resonance, high resolution mass spectrometry, hyperspectral reflectance, and integrative chemometrics analysis

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The olive quick decline syndrome (OQDS) is the disease that, in the past decade, caused severe damages to the ancient olive trees of Apulia region, in Southern Italy. *Xylella fastidiosa* (Xf) was ascertained as the main agent of OQDS. During the infection period, until the complete decline, the trees go through phenotypic and metabolic changes, as the latter was demonstrated by a non-targeted metabolomics study on Xf-infected olive plants. Such a study used nuclear magnetic resonance (NMR) spectroscopy and high-resolution mass spectrometry (HRMS) to propose a set of metabolites playing a possible role as biomarkers for Xf-infections, in which higher amounts of malic acid, formic acid, mannitol, and sucrose were higher in Xf-infected plants than in Xf-uninfected ones. In contrast, Xf-infected plants revealed slightly lower amounts of oleuropein. As a follow up, the Xf-infected olive plants were studied using hyperspectral reflectance (HSR) aiming at selecting a wavelength region(s) diagnostic for Xf-infections. This integrative approach, successfully applied before, was applied encompassing NMR, HRMS, HSR, and chemometrics. The susceptible variety Cellina di Nardò was studied for artificial infection by *X. fastidiosa* subsp. *pauca* ST53, also known as De Donno strain. Plants were grown in a thermally-controlled environment and co-inoculated with some xylem-inhabiting fungi known to occur with OQDS. Leaves were subjected to HSR acquisition while their extracts were analysed by both NMR and HRMS and the acquired spectra were analysed using chemometric techniques. Then, the covariance matrices between those methods were used as a guide to connect HSR spectral features with some diagnostic NMR and HRMS signals. Furthermore, such matrices revealed different ranges of wavelength-regions with different association levels to the corresponding metabolites, which were of few regions within the visible range of 420-520 nm, 570-720 nm, and more within the near-infrared range of 1000-1830 nm.

Keywords: Hyperspectral Reflectance, Metabolites, Chemometrics Analysis

Use of indicator plants for the early detection of *X. fastidiosa*

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Xylella fastidiosa (Xf) is considered to be one of the most serious threats to plant health globally (1, 2, 3). Movement of plants for planting has been estimated as the most important pathway for entry of Xf into Europe, especially when present without symptoms (3). The significance of this pathway is enhanced by the enormous host range of Xf and the high volumes of plants for planting imported from countries where Xf was known to occur. To control further the spread of Xf, it is crucial that newly infected sites are quickly detected (3). Experimental host plants have been previously used for studying the plant-*Xylella fastidiosa* interactions (4), as being good indicators of pathogen infection, allowing systemic colonization and the expression of disease symptoms. Seven indicator plants were selected for experiments, namely: *Nicotiana tabacum* cv. SR1 (Petite Havana), *Catharanthus roseus* cv. Cooler Peppermint, *Polygala myrtifolia* cv. Bibi Pink, *Prunus avium*, *Prunus domestica*, *Nerium oleander* cv. Rojo Red, *Coffea arabica* cv. Nana. These indicator plants were artificially inoculated (in repeated rounds of inoculation) with two Xf subsp *pauca* and one Xf subsp *multiplex* isolate. Two independent experiments have been performed. Experiments have been completed in 20-22 months after the first artificial inoculations. Final sampling was performed from each individual plant in a destructive way, and tested by the real time PCRs (Harper et al., 2010; erratum 2013 and Quyang et al., 2013) to check for the presence of Xf. Our results demonstrated that mainly *P. myrtifolia* and alternatively, *N. oleander* are two very efficient indicator plants, under the restriction that the initial infection occurs during the spring and summer period. However, when the initial infection occurs during the autumn and winter period, Xf could not be confirmed in the majority of the individual plants included in this experiment, regardless of the plant species and the Xf isolate used for the inoculation.

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Diagnostic pipeline and large-scale monitoring: the experience in the Apulian outbreak

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In Apulia (southern Italy) since 2017 one of the largest monitoring campaign in EU is ongoing, with an average number of 100.000 samples tested per year in the framework of the official monitoring program for *Xylella fastidiosa*, in the demarcated and *Xylella*-free areas. More than 85% of the samples were from olive trees, the remaining from almond, oleander and other host plants. Before the promulgation of the EU Regulation 2020/1201, the diagnostic program was organized with two levels of checks. Samples were first screened in 4 regional laboratories by ELISA, with each laboratories processing 300-600 samples/day. Samples yielding positive or doubtful ELISA-results along with 3-5% of the negative samples, were re-tested by qPCR as confirmation tests and for verifying the lab performance for the serological tests. An average of 8.000 sample/year were re-tested by qPCR. Using this large dataset of samples (double tested by ELISA and qPCR) we determined the diagnostic specificity and sensitivity of the test used, and assessed the influence of other factors (period of sampling, origin of the samples). Overall, less than 0.03% of the ELISA-tested samples could not be assigned as negative/positive (i.e. undetermined) and thus re-tested by qPCR, yielding in the majority of the cases (>85%) negative qPCR-results. The diagnostic sensitivity and specificity calculated on the panel of ELISA-negative/positive samples re-tested in qPCR, approx. 26.000 samples, were 94% and 97%, respectively. These values did not vary significantly when the results were analyzed based on the period of sampling or location of the sampled trees. In conclusion, under the specific scenario characterizing the Apulian epidemics (olive predominant host plants, climatic conditions) this diagnostic workflow had allowed to screen with good diagnostic accuracy a relevant number of samples/year. Given the need to replace ELISA with qPCR tests, the capability of the laboratories to process the samples is now reduced (approx. half samples/day) and other possibilities will be considered for the implementation of the upcoming monitoring campaign (i.e testing pooled samples) to ensure testing the same amount of samples.

Keywords: Detection, accuracy, large-scale monitoring

Capturing efficacy assessment of antibodies against *Xylella fastidiosa* through Surface Plasmon Resonance

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Xylella fastidiosa is among the most dangerous plant pathogenic bacteria, worldwide causing a variety of diseases with huge economic impact on agriculture and environment. A surveillance tool, ensuring the highest possible sensitivity enabling the early detection of *X. fastidiosa* outbreaks, would be of paramount importance. Fundamental to the development of such a detection system is the availability of high affinity and specific antibodies to the bacterium. So far, a variety of plant pathogen biomarkers were studied by means of Surface Plasmon Resonance (SPR), but none of them involves *X. fastidiosa*. In this study SPR has been proposed to assess the optimized biofunctionalization protocol of gold surfaces with anti-XF, validating their capturing efficacy against *X. fastidiosa*. Selectivity is guaranteed by bio-functionalizing the gold transducing interface with polyclonal antibodies for *X. fastidiosa* and it was assessed by means of a control experiment involving the non-binding Burkholderia phytofirmans bacterium. Remarkably, a limit of detection as low as 105 CFU/mL was achieved by transducing the direct interaction between the bacterium and its affinity antibody, which is comparable to the label-needing ELISA gold standard. Moreover, the binding-affinity between polyclonal antibodies and the X. fastidiosa bacteria has been also evaluated, obtaining an affinity constant of 3.5·107 M-1, comparable with those given in the literature for bacteria detection against affinity antibodies. The study is therefore a preliminary development of a reliable cost-effective process to successfully bio-functionalize a gold surface, eventually suitable as gate electrode in wide-field bioelectronic sensors, for ultra-sensitive detection of *X. fastidiosa*.

Xylella fastidiosa field detection combining Loop-Mediated Isothermal Amplification (LAMP) with Long-read Nanopore sequencing

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Xylella fastidiosa (XF) is a phytopathogenic bacterium leading to losses and damage to large agricultural and forestry areas causing great economic and environmental impacts. This quarantine organism (A2 list, European and Mediterranean Plant Protection Organization (EPPO)) has its impact determined by the subspecies, the host, the vectors transmission, and environmental factors, but an early detection will help farmers to take preventive measures by reducing impact, costs, and damage. In this line, Loop-mediated isothermal amplification (LAMP) and Oxford Nanopore Technology (ONT) are two methods that are becoming important due to their rapidity, sensitivity and their portable format applied to various environments and for plant pathogen detection. This present work proposes to make the first step towards developing a simple, accurate and sensitive real time detection tool to be used in the field. This consists in coupling LAMP with a 3rd generation sequencing technology, the ONT MinION MK1c device. LAMP allows, without prior DNA extraction, a portable, simple, fast, and inexpensive sensitive and specific detection and ONT MinION MK1C assures the diagnostic accuracy. For XF LAMP detection, we used previously designed primers for rimM gene and newly designed primers for at least Multi-Locus Sequence Typing (MLST) of three housekeeping genes: cysG, malF and rpoD. Currently, our preliminary results show that we can detect and confirm the XF presence using rimM gene primers. This method allows to reduce diagnosis from weeks to hours. This study is the first step towards applying fast, accurate and sensitive methods to prevent plant diseases in an early stage, avoiding pathogen field dissemination.

Enhancing the UK diagnostic capabilities for *Xylella fastidiosa*

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Reliable and accurate diagnostic tools are critical to enable effective response to *Xylella fastidiosa* in the event of an outbreak. Current testing procedures including different real-time PCR assays are being validated and optimised for use by accredited laboratories in the UK. Informal DNA based proficiency tests for real time PCR assays completed by four institutions gave consistent results.

Three host species (*Lavandula dentata, Nerium oleander, Coffea arabica*) were inoculated with three subspecies of *Xylella fastidiosa* and kept in two different glasshouse environments (25°C and ambient tempreature) where rates of colonisation and symptom expression were monitored to inform future sampling strategies. Symptoms that could be due to *Xylella* infections were not always associated with positive qPCR samples; oleander and coffee plants were successfully infected and, although most positive samples were close to inoculation points, some oleander samples taken up to 10cm away from the points were also positive showing that the infection had progressed.

Other approaches, such as detection of volatile organic compounds (VOCs), are being assessed to target sampling. A proof of principle study using coffee plants showed that a large amount of VOCs can be retained on Mono Traps (a portable sorptive solid media) and a number of compounds were different between control and *Xylella* infected plants.

Methods for identifying subspecies and strains are being developed to enable tracing of infection sources. Concatenated MLSA sequences were collected for all publicly available genomes of *X. fastidiosa* and trees built to visualise relationships between clonal complexes. Using public sequence data of over 200 genomes, a species-level cgMLST scheme was created for *X. fastidiosa* as well as schemes for three individual subspecies. As well as application to whole genomes, the resolution provided by the cgMLST approach has also been shown to be informative when applied to sparser data from infected plant metagenomes.

Keywords: detection, sampling, sequencing

Xylella fastidiosa: Imminent Risk to Food Security in Near East and North Africa Region

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The first report of *Xylella fastidiosa* (XF) in southern Italy in 2013 has triggered great anxiety for agriculture business in the Mediterranean region. The presence of such a pathogen poses a serious threat to crop production, food security and employment in the region's rural areas. The favourable climatic conditions, the abundant insect vectors and the wide distribution of host plants would foster the spread of the disease. Propagation materials are considered the most likely means of long-distance spread. Thus, the risk for Near East and North Africa (NENA). For these reasons, such a "silent killer" requires an effective surveillance system to be operational before significant damage can be noticed, to act as an early warning system. Food and Agriculture Organisation (FAO) launched a regional project to support NENA countries in enforcing preventive measures against the introduction and spread of XF, FAO has provided training workshops, seminars, laboratory equipment, technical documents, awareness materials, and recently evaluated the potential socio-economic impact of the diseases on NENA countries. Support is still needed in the following areas: i). Policy support: by Enforcing and reforming the phytosanitary systems; and updating national contingency/action plans by providing the needed tools for implementation. ii). Research: Identification of potential insect vectors of XF; Prediction of the effect of climate change on disease development; Building regional laboratory network to harmonize protocols for detection; and Identification and development of local cultivars with inherited genetic resistance/tolerance for XF. Capacity building: Plan and implement an efficient and cost-effective surveillance programme; Development of the capacity of phytosanitary officers in inspection and sampling, national/regional platform for knowledge sharing; Improvement of technical capacity of plant quarantine at entry and post entry points; Development and application of innovative surveillance methods; and Improving capacities of farmers through farmer field schools.

POSTER SESSION:
Endophytic microbial resources and their potential applications for *Xylella fastidiosa* control

Title	Presenter	DOI
Culture and metagenomic approaches for the identification of olive xylem microbial communities as a biological control tool to cope against *Xylella fastidiosa* infection*	**Anguita-Maeso M**, Institute for Sustainable Agriculture, Spanish National Research Council, Córdoba (ES)	**https://doi.org/10.5281/zenodo.4671712**
Mapping *Xylella fastidiosa* infection and xylem microbiome composition on olive tree branches.	**Anguita-Maeso M**, Institute for Sustainable Agriculture, Spanish National Research Council, Córdoba (ES)	**https://doi.org/10.5281/zenodo.4671892**
Antagonism capacity of endophytes isolated from olive cvs in Apulia region	**Hanani A,** University of Palermo and CIHEAM of Bari (IT)	**https://doi.org/10.5281/zenodo.4672069**
Fungal metabolites for the biocontrol of *Xylella fastidiosa*	**Cimmino A,** Department of Chemical Sciences University of Naples Federico II, Naples (IT)	**https://doi.org/10.5281/zenodo.4683060**
Zinkicide nanoformulation efficacy to mitigate xylem limited *Xylella fastidiosa* strains in tobacco and blueberry	**Shantharaj D**, Department of Entomology and Plant Pathology, Auburn University, Auburn (US)	**https://doi.org/10.5281/zenodo.4683082**
Heterologous expression of CECMEL11 bactericidal peptides against *Xylella fastidiosa* in *Nicotiana benthamiana* plants	**Montesinos L**, University of Girona, Girona (ES)	**https://doi.org/10.5281/zenodo.4683098**
Development of natural products against the emerging plant pathogen *Xylella fastidiosa*	**Robledo M,** Biomar Microbial technologies, León, Spain and Universidad de Cantabria, Santander (ES)	**https://doi.org/10.5281/zenodo.4683108**
Looking for olive XF-resistance under high natural selective pressure in Salento	**La Notte P,** Institute for Sustainable Plant Protection, CNR, Bari (IT)	**https://doi.org/10.5281/zenodo.4683117**
Evaluation of the sexual compatibility between Leccino and FS17	**Roseti V,** University of Bari Aldo Moro, Department of Soil, Plant and Food Sciences, Bari (IT)	**https://doi.org/10.5281/zenodo.4683134**
Screening of a novel biological control agent targeting the phytopathogen *Xylella fastidiosa**	**Mourou M,** Università Degli Studi Della Tuscia, Dipartimento di Scienze Agrarie e Forestali (DAFNE), Viterbo (IT)	**https://doi.org/10.5281/zenodo.4683142**

In vitro and in vivo effects of quaternary ammonium compounds on *Xylella fastidiosa* subsp. *pauca* infecting olives	**D'Attoma G**, Institute for Sustainable Plant Protection, National research Council (IT)	**https://doi.org/10.5281/zenodo.4683351**
An interdisciplinary approach to prevent *Xylella fastidiosa* outbreaks	**Farigoule P,** AgroParisTech, Paris, France & CBGP, INRAE, CIRAD, IRD, Montpellier SupAgro, Univ. Montpellier, Montferrier-sur-Lez (FR)	**https://doi.org/10.5281/zenodo.4683361**
Peptides of synthetic and microbial origin with antimicrobial and antibiofilm activity against *Xylella fastidiosa**	**Moll L,** Laboratory of Plant Pathology, Institute of Food and Agricultural Technology-CIDSAV-XaRTA, University of Girona (ES)	**https://doi.org/10.5281/zenodo.4683372**
Bacteriophage for treatment and prevention of *X. fastidiosa* infection in grapevines (Pierce's Disease)	**Kinkhabwala A**, A&P Inphatec, Palo Alto, California (US)	**https://doi.org/10.5281/zenodo.4683402**

**abstracts selected through the Young researchers' initiative are reported in the oral presentations section only.*

Mapping *Xylella fastidiosa* infection and xylem microbiome composition on olive tree branches

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The plant-pathogenic bacterium *Xylella fastidiosa*, widely distributed in the Americas and with several recent outbreaks in EU, emerged as a serious threat to olive crop and the typical Mediterranean landscape. This xylem-limited plant pathogen spreads by xylem sap-feeding insects, causes symptoms of marginal leaf scorch, leaf chlorosis, defoliation, and a general decay in olive trees. Research on plant-associated microorganisms has gained importance in the last decade as a key component in plant health. However, the role of the xylem microbiome and its contribution to plant health is still scarce. Therefore, this work is focused on mapping *Xylella fastidiosa* infection and deciphering the changes in the composition of microbial communities on symptomatic and asymptomatic olive tree branches. Three olives trees of cultivar Grappolo growing at Sao Paulo state (Brazil) were selected and within each tree three main branches were sampled showing different severity of symptoms development; one branch presenting typical *X. fastidiosa* symptoms of pathogen infection in contrast to the other two branches that were asymptomatic. Xf infection was verified by qPCR. Leaves and stem samples were collected in a gradual scale above and below the symptomatic branches. Chips extracts of xylem tissue from its stems and leaf veins and petioles were used for DNA extraction to undergo metagenomic analysis (NGS) for microbial composition estimation and to diagnose the presence of the pathogen by real-time PCR, respectively. These results will help to understand the occurrence of step-wise changes in olive xylem microbiome as a consequence of *X. fastidiosa* infection and determine which microorganisms respond faster or can be associated to a symptom progression on infected trees.

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Antagonism capacity of endophytes isolated from olive cvs in Apulia region

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Endophytes can play an antagonistic role in controlling plant vascular diseases. However, this depends on their efficiency of establishment in the plant's internal sphere and their ability to stimulate the plant's defense mechanisms. The present study aimed to evaluate the antagonism capacity of endophytes isolated from olive cvs (*Bacillus subtilis, Pantoea agglomerans, Paraconiothyrium brasiliense*) on plant colonization and production of plant defense-related genes in aerial tissues. After inoculation of the selected antagonists on 'Leccino' and 'Cima di Mola' plants in the greenhouse, diagnostic tests for their detection were carried out for two months period using Real-time PCR. Production of plant defense-related genes in aerial tissues of inoculated plants was evaluated. All endophytic agents were successfully inoculated into the cv 'Leccino' and 'Cima di Mola' where they multiplied and spread. *Bacillus subtilis* showed a high stability and persistence at a concentration of 104 CFU/ml, while *P. agglomerans* at lower levels <102 CFU/ml. *Paraconiothyrium brasiliense* was constantly found at a high colonization rate (>70%) in cultured stem discs of both cultivars. The expression of defence-related genes was detected in plants inoculated with *B. subtilis*, but not in those inoculated with *P. agglomeransa* and *P. Brasiliense*. Our findings suggest the potential direct and indirect antagonism effect of *Bacillus subtilis* in olive cvs that could be investigated for biocontrol against Xf (ST53).

Keywords*: Bacillus subtilis, Paraconiothyrium brasiliense*, defense genes

Fungal metabolites for the biocontrol of *Xylella fastidiosa*

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Xylella fastidiosa is a Gram-negative bacterium causing devastating diseases of many plants, including important crops as grapevine, peach, citrus, almond, coffee and pear. In the last decade, X. fastidiosa has devastated olive heritage in Apulia region (Italy) inducing a severe disease called "Olive Quick Decline Syndrome" The disease also infected millennial olive plants. The bacterium was introduced in this region from abroad and rapidly spread causing heavy economical losses and cultural and social damages. The eradication of infected plants in the restricted area at beginning of infection could be an effective approach to eliminate the bacterium. Unfortunately, this method was too late and partially applied. Studies carried out successively demonstrated that a zinc-copper-citric acid bio-complex allowed a consistent reduction of the infection on olive trees. The use of synthetic bactericides was discouraged as their use could cause resistance in the host plants and because of the long term impact of residues in agricultural products with a risk to human and animal health. Thus, a valid alternative could be the use of natural antibacterial metabolites. Some year ago, radicinin, a pihydropyranopyran-4,5-dione, was proposed as natural pesticide to control the infection of *X. fastidiosa* causing grapevines (*Vitis vinifera* L.) Pierce's disease. Recently, radicinin together with some analogues were isolated from Cochliobolus australiensis, a fungus proposed as mycoherbicide to control buffelgrass (*Cenchrus chliaris*) an invasive weed in North America. The fungal production of radicinin was very low and this prompted the development of an efficient and ecofriendly total synthesis that afforded in good yield its biosynthetic precursor 3-deoxyradicinin. Further studies showed that 3-deoxyradicin displays phytotoxic activity against buffelgrass and antibacterial activity against *X. fastidiosa* comparable with those of radicinin. These results allowed us propose both radicinin and 3-deoxyradicinin as potential natural pesticides which, conveniently bioformulated could be used to control of *X. fastidiosa*.

Zinkicide nanoformulation efficacy to mitigate xylem limited *Xylella fastidiosa* strains in tobacco and blueberry

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Blueberry leaf scorch disease caused by *Xylella fastidiosa* causes important economic losses in the US. There is growing interest in employing nanoformulations for managing plant diseases due to their efficacy with particle size. In this study, we evaluated the efficacy of the ZnO based nanoformulation Zinkicide (ZnK) against *X. fastidiosa*. In vitro assays showed that ZnK minimum inhibitory concentration, MIC50, and MIC90 was 50 ppm and ≥ 60 ppm respectively. Initially, ZnK efficacy was tested in the model tobacco pathosystem infected with *X. fastidiosa* subsp. fastidiosa TemeculaL. ZnK added as soil drenches at an optimum cumulative concentration of 2500 ppm showed bactericidal activity by reducing ~1000-fold *X. fastidiosa* TemeculaL population. Subsequently, ZnK was evaluated in the greenhouse using blueberry infected with *X. fastidiosa* subsp. multiplex AlmaEm3. Preliminary results showed that an optimum non-phytotoxic ZnK concentration of 2000 ppm led to bactericidal activity, causing a slow progression of *X. fastidiosa* AlmaEm3 growth in the xylem, resulting in overall lower disease incidence and reduction in bacterial population by ~10-fold. Evaluation of systemic mobility of ZnK in other hosts as well as identification of non-phytotoxic concentrations will allow application of this management strategy transferrable to other hosts to limit *X. fastidiosa* outbreaks.

Keywords: Zinkicide, blueberry, *Xylella fastidiosa*

Heterologous expression of CECMEL11 bactericidal peptides against *Xylella fastidiosa* in *Nicotiana benthamiana* plants

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Xylella fastidiosa (Xf) diseases have emerged in recent years as several outbreaks of considerable intensity and extension in Europe. Their management is currently based on the use of pathogen-free propagation plant material, quarantine and eradication measures, and vector control. However, no cure of the disease exists up to now. Antimicrobial peptides have been identified as potential candidates for plant disease control, and have demonstrated a strong potential for the control of Xf. Specifically, it has been reported that the BP100 derivative peptide, BP178, synthetized for being expressed in plants, show high in vitro antibacterial activity against a collection of Xf strains. Although the application of bactericidal compounds to control xylem and phloem limited bacterial diseases has been tested by spray or trunk injection, the production of transgenic plant lines expressing antimicrobial peptides has been reported as promising alternative in the control of Xf. We have developed a transient-expression system based on Potato virus X (PVX) in *Nicotiana benthamiana* plants, for the production of the BP178 peptide in plants and evaluation of their potential in controlling Xf. The BP178 sequence was engineered in a PVX viral vector to be expressed in fusion with the NH2 Coat protein terminus. The release of the peptide occurs by the proteolytic activity of a Foot and Mouth Disease Virus 2A sequence. Western blot analysis showed that the BP178-CP fused proteins were expressed in *N. benthamiana* plants infected with the recombinant PVX-BP178-CP. Co-inoculations of *N. benthamiana* plants with PVX-BP178-CP and Xff strain IVIA 5387.2 are ongoing with initial results showing efficacy in controlling the pathogen over time.

Keywords: antimicrobial peptide, PVX

Development of natural products against the emerging plant pathogen *Xylella fastidiosa*

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Biomar (http://biomarmt.com/) is a biotech company located in León (Spain) fully devoted to marine microbiology and natural products (NPs) chemistry. With one of the largest microbial libraries in the world, Biomar develops natural solutions for different industries in collaborations with leading companies in the field. Our agriculture projects involve biostimulants, bioherbicides, biofungicides and bioinsecticides from microbial origin. All of them are focused on NPs obtained by microbial fermentation, therefore from sustainable biotechnological sources. Among others, our company enabled the identification of new conjugation and biofilm formation inhibitors, which are under evaluation for potential therapeutic use in different applications. Development of efficient methods for *Xylella fastidiosa* control is a worldwide priority. Recently, Biomar has taken an active part in a platform to search for innovative approaches and solutions to the disease caused by *X. fastidiosa*. The project aims at developing natural growth and biofilm's altering compounds to prevent or treat *X. fastidiosa* plant infections by characterizing already reported active molecules, a collection of extracts and underexplored natural products. First, we have developed and validated a reliable high-throughput screening assay against *X. fastidiosa* (Xf-HTS). Currently, part of our compounds and extracts libraries are being screened using our Xf-HTS and first promising candidates and active analogs have been chemically identified. Efficacy will be also evaluated in vitro under host-mimicking conditions using microfluidic devices. Further development of this project will require greenhouse and field trials with host plants and insect vectors that will be complemented by a combination of analytical tests, and genetic and molecular approaches to try to develop an effective treatment against this devastating disease.

Looking for olive XF-resistance under high natural selective pressure in Salento

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Several success stories in plant pathology indicate that the use of plant genetic resistance is the most effective, durable and environmentally/economically sustainable protection system for the coexistence with harmful organism whenever curative methods lack. In the epidemic of *Xylella fastidiosa* ST53 in Salento, the cultivars Leccino and FS17 showed interesting resistant traits. This important finding, even if allowed the replanting of olives in infected areas, is considered a starting point being highly desirable to find a wider range of varieties to avoid a dangerous genetic uniformity and to offer alternatives to the growers and oil producers. For this purpose in Salento, a large-scale program for testing the susceptibility of Italian and Mediterranean olive germplasm started and the first long-lasting breeding activities for the resistance/tolerance were initiated. Due to the urgency, the selection of productive plants derived from uncontrolled pollination and seed dissemination was identified as a third approach aimed to save the time of long olive juvenile period and enlarge the genetic variability to be explored looking for resistance or tolerance directly under high selective pressure of inoculum in the infected area. Furthermore, each seedling plant, derived mainly from parentals of the local cultivars, is potentially a new candidate "autochthonous" variety. After 3-years of activity in the framework of the regional project ResiXO and thousands of plants observed/tested, the activities of morphological/technological characterization, as well the pathogenicity tests by pathogen inoculation in controlled conditions started on a panel of candidate potentially resistant genotypes. Positive feedbacks are also coming from a newborn initiative called Xylor (Xylella Olive Resistance), contrived as a citizen science approach launched in September 2019 to involve the local community in the exploration of a huge territory to find plants asymptomatic or with mild symptoms in the abandoned and dying olive orchards.

Keywords: *Xylella*, olive, resistance.

Evaluation of the sexual compatibility between Leccino and FS17

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Following the outbreak of the plant bacterium *X. fastidiosa subsp*. Pauca ST53 in 2013, the "Olive Quick Decline Syndrome" has wreaked havoc in Italy's Puglia region, wiping out hundred thousands of olive trees throughout the southernmost provinces. Over the years, various degrees of susceptibility and symptom expression have been confirmed. Two *Xylella*-resistant olive varieties, Leccino and FS17 or Favolosa, have been identified and authorised for planting within the Infected Area in a move to boost the revival of the hard-hit olive oil industry, as per Resolution of the Regional Council N. 200 of 5 February 2019, and in keeping with EU Plant Protection Regulations. In order to hasten fruit set in newly-established orchards, given that self-sterility and/or self-incompatibility are commonly reported in olive and all potential pollinisers of the self-sterile Leccino are known to be susceptible to the bacterium, a trial was conducted in 2019 and 2020 to assess sexual compatibility of FS17 (self-compatible) and Leccino. A Leccino orchard was identified in the countryside of Gagliano del Capo where 6 plants in the preflowering stage were randomly selected. On each mother plant 4 combinations were tested with 2 replicates each: non-pollinated Leccino; Leccino exposed to free pollination; Leccino (seed stock) x Leccino (with pollen originating from plants raised in the same plot); Leccino x FS17. Fruits were counted 30 days after treatment, following the physiological fruit drop of July and at harvesting. The findings demonstrate that FS17 may be considered a polliniser of Leccino, as the average fruits exceed the percentage (3%) of treated flowers, in line with yield data available in the literature. Their sexual compatibility is based on genetic factors as well on phenological factors due to a partial overlapping of the flowering periods with Leccino that reaches the full flowering around one week later FS17.

Keywords*: xylella*, olive resistance, cross-pollination

In vitro and *in vivo* effects of quaternary ammonium compounds on *Xylella fastidiosa* subsp. pauca infecting olives

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Quaternary ammonium compounds (QACs) are cationic surfactants and antimicrobials whose effectiveness has been previously proved against numerous bacteria, e.g. *Pseudomonas aeuriginosa*, *Staphylococcus aureus*, and *Escherichia coli*. The antimicrobial activity involves interaction with cell membranes, disruption of membrane integrity, and leakage of cellular content. Although with a different mode of action, timing and doses. This study aims to investigate the impact of ammonium chloride on the growth performance of *Xylella fastidiosa* strain De Donno and explore its potential use in the field to mitigate the impact of the infections in olives. Three concentrations of ammonium chloride (0.25-0.5-1%) were tested in liquid medium to evaluate the influence on the bacterial viability after 3 days and 6 days of growth. Cristal violet assay was used to determine the effect of the QAC on the adhesion of the bacterium and its ability to form biofilm. Gradual growth inhibition was observed with increasing concentration of ammonium chloride in the growth medium. The growth inhibition effect is evident on planktonic cells, but is significantly more pronounced on biofilm-forming cells due to the peculiar adhesiveness of the *Xylella fastidiosa* strain De Donno. Field experiments started only recently (2019-2020) and included applications of ammonium chloride 4 times/year from March to October, in olive groves with different incidence of the infections. A general increase of the vegetation mass and an attenuation of the wilting and desiccation phenomena (particularly in the bottom portion of the canopies) was observed in all treated trees, although not supported by statistically significance difference. Quantitative PCR tests on the experimental plants did not reveal any difference in the population size of treated and untreated plants. In conclusion, ammonium chloride showed in vitro effects on bacterial growth/biofilm formation, and an attenuation of symptoms severity was detected, although prolonged experiments and observations are needed.

An interdisciplinary approach to prevent *Xylella fastidiosa* outbreaks

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Since the detection of *Xylella fastidiosa* (Xf) in Italy, European research interest into this bacterium has considerably increased. Research mainly focusses on Xf biology, evolution and epidemiology. However, managing the spread of Xf also requires the mobilization of other actors, whose action supports research driven strategy. Indeed, people who are directly impacted (arboriculturalist, winegrowers) must be involved to slow down the spread of Xf. We propose an interdisciplinary work that combines the production of knowledge in ecology with the analysis of the construction, use and diffusion of this knowledge to stakeholders using social studies of science and technology practices. In ecology, field observations, statistical and biomolecular analyses are conducted to characterize plant - insect vector and insect vector - Xf interactions. In sociology, an analysis of scientific production is carried out through the implementation of participatory observation to understand how scientific activities are linked with epidemiological surveillance. In addition, an analysis of the preparedness of stakeholders (professionals from agriculture, research and institutions involved in the French epidemiological surveillance system) based on semi-directive interviews is implemented. This interdisciplinary study, whose approach and first results will be presented has two objectives i) to propose prophylactic and control methods to slow down or prevent the establishment of Xf in the uncontaminated territories and ii) to provide recommendations for the dissemination and implementation of these practices with an evaluation of the preparedness in France. This approach could also lead to a reflexive understanding of how sciences and public management might be articulated to understand Xf disease as a socio-pathosystem.

Bacteriophage for treatment and prevention of *X. fastidiosa* infection in grapevines (Pierce's Disease)

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Few methods for controlling and treating Pierce's Disease (PD) have been available, with efforts historically focused on controlling the sharpshooter vector (e.g., insecticides) or roguing seriously ill vines, both of which have demonstrated only limited success. However, an option that reduces PD in grapevines is now available, XylPhi-PD.™ XylPhi-PD is a novel biological treatment for PD that is approved for use in organic production. This breakthrough technology was developed exclusively for viticulture. XylPhi-PD contains a cocktail of viral bacteriophages (bacteria-killing viruses) that enter and destroy *X. fastidiosa* bacteria. XylPhi-PD can be flexibly applied as a preventative to protect growing vines under disease pressure, or as a curative after disease symptoms become visible. Multiple field studies assessed the efficacy of XylPhi-PD for the treatment or prevention of Pierce's Disease (PD) in vineyards, when used in accordance with label Directions for Use. In a 2015 Texas A&M study with natural infection exposure, 3 monthly XylPhi-PD treatments significantly reduced PD incidence 44% vs controls. In an ongoing 4-site study (up to 2 years of treatment in leading North Coast California commercial vineyards with high PD pressure), XylPhi-PD treatment of diverse wine varietals prompted reductions in visual PD symptoms of 60%, which was confirmed by qPCR. XylPhi-PD has consistently demonstrated significant efficacy across differing conditions and locales. These favorable outcomes distinguish XylPhi-PD as a targeted and effective strategy for protecting valuable vineyards against PD.

POSTER SESSION:
Social sciences and communication

Title	Presenter	DOI
A socio-semantic analysis of the research domain on *Xylella fastidiosa*. Structure and scientific dynamics	**Barbier M.,** INRAE – UMR LISIS, Marne-la-Vallée (F)R	**https://doi.org/10.5281/zenodo.4683429**
Media and scientific literature monitoring of *Xylella fastidiosa* using the MEDISYS platform*	**Campese C.,** Animal and Plant Health Unit, European Food Safety Authority (EFSA), Parma (IT)	**https://doi.org/10.5281/zenodo.4683526**
The role of citizen scientists in preparing for a national response to the invasive insect-transmitted plant pathogen *Xylella fastidiosa*	**Pérez-Sierra A.,** Forest Research, Farnham (UK)	**https://doi.org/10.5281/zenodo.4683574**

**abstracts selected through the Young researchers' initiative are reported in the oral presentations section only.*

A socio-semantic analysis of the research domain on *Xylella fastidiosa*. Structure and scientific dynamics

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This poster intends to offer a socio-semantic overall view of the research domain on *Xylella fastidiosa* as a major quarantine pest with worldwide expansion. This scientometric analysis is applied on a corpus of bibliographic notices, which has been delineated in the Web of Science database with a query based on the reference to *Xylella fastidiosa* bacteria as a matter of scientific enquiry and as models for phytopathology. Analysis are conducted thanks to the CorTexT Platform, an open science capacity to run various type of textual and network analysis which belong to the RISIS European Infrastructure for science and innovation policies (https://www.risis2.eu/). The purpose of this analysis is to deliver to researchers and policy-maker a retrospective account of the constitution and development of the research domain on *Xylella fastidiosa*. This account is based on the complementary visions and knowledge of authors' discipline: computational social science, phytopathology, ecology and genomics.

Methodology. Based on the co-word analysis tradition in Scientometrics (Callon et al., 1983; Waltman, Noyons et al., 2010) and advanced methods in quantitative sciences studies (Cambrosio et al., 2020) our quali-quantitative approach of scientific literature is based on some already well-documented methods of homogeneous and heterogeneous networks analysis and their visualization with Louvain clustering algorithm. We mobilize the portfolio of scripts and algorithms that are proposed on the open platform CorTexT (Barbier and Cointet, 2021). Harnessing a methodology already elaborated in a previous research about ecosystem services (Tancoigne et al., 2014) or Nanotechnology (Kahane et al. 2015) or synthetic biology (Raimbault et al., 2016), we establish the structural composition of this particular research domain with clustering methodology.

Results. A common analysis of fields like co-authors, keywords and cited-reference networks deliver a global and structural account of the domain a first layer of descriptive results. Then, using the projection of a third variable (authors' countries; journals) with a contingency matrix analysis, we show the existence of national research engagements and almost geopolitics of xyl. fast. research, because of historical investments of scientific activities on the main plant diseases induced by this bacterium. A third result is produced while using bibliographic coupling to cluster the results of a terminological extraction of abstracts; we establish the socio-epistemic features of the domain, and the disciplinary or interdisciplinary lineaments of its composition. This approach enables to deepen the understanding of the research domain despite its polarities on wine grapes, citrus and olive tree. Finally, thanks to evolutionary approach of clustering extracted N-Grams (Chavalarias and Cointet, 2013) we characterised the scientific dynamic of xyl. fastidiosa research domain in the long run. Conclusion. Our analysis establishes the continuous polarization of scientific exploration through time but also the latest scientific achievements, corresponding to new issues, and the late outbreak in Puglia. This perspective enables to identify the European specificity that is emerging with previous outbreaks.

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The role of citizen scientists in preparing for a national response to the invasive insect-transmitted plant pathogen *Xylella fastidiosa*

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In recent years there has been an increase in the number of pests and diseases threatening plants in the UK. Active surveillance by official regulatory bodies is key for their early detection and to enable their management. However, additional surveillance by citizen scientists can support national surveillance programmes as they widen the area surveyed beyond points of entry and the plant trade. *Xylella fastidiosa* was detected for the first time in Europe in 2013 and has been detected in France, Italy, Portugal and Spain. The pathogen has not been detected in the UK although the spittlebug *Philaenus spumarius*, the main vector of *X. fastidiosa* identified in Europe is common and widespread. In 2018 the BRIGIT project was funded to identify strategies to mitigate the impact of *X. fastidiosa* and to increase preparedness should the pathogen be introduced.The BRIGIT project has aimed to raise awareness and engage with the public to gather information related to the distribution and feeding hosts of *P. spumarius* in Britain. Working with stakeholders and the broader public, BRIGIT has developed and optimised a range of resources, including information datasheets, databases and survey tools to enable the identification and reporting of insect vectors and the plant species that are known hosts for *X. fastidiosa*. A series of face-to-face and online training events have raised the overall awareness of *Xylella*, its insect vectors, regulations and biosecurity with professional and citizen scientists. Press, social media and multimedia outputs have promoted the citizen science surveillance and resulted in over 24,000 records being submitted to map insect vectors and plant host species in the UK. We explore how our engagement on *Xylella* could lead to greater citizen involvement in national surveillance and present some preliminary feedback on our attempts to improve awareness and influence better biosecurity behaviours.

Keywords: surveillance, citizens, awareness

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