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PEST ORGANISMS THREATENING EUROPE

XF-ACTORS

XYLELLA FASTIDIOSA ACTIVE CONTAINMENT
THROUGH A MULTIDISCIPLINARY-ORIENTED RESEARCH STRATEGY

3RD JOINT ANNUAL MEETING

BOOK OF ABSTRACTS

AJACCIO (FRANCE), 28–30 OCTOBER 2019

PALAIS DES CONGRÈS, QUAI L'HERMINIER
AJACCIO, FRANCE

THE EVENT WAS PART OF THE SECOND SCIENTIFIC CONFERENCE ON ONGOING RESEARCH INTO *X. FASTIDIOSA* CO-ORGANISED WITH EUROPEAN FOOD SAFETY AUTHORITY (EFSA), H2020-MSCA-RISE PROJECT 734353 'CURE-XF', COST ACTION CA16107 'EUROXANTH', FRENCH NATIONAL INSTITUTE FOR AGRICULTURAL RESEARCH (INRA), FRENCH AGENCY FOR FOOD, ENVIRONMENTAL AND OCCUPATIONAL HEALTH AND SAFETY (ANSES), EUPHRESCO, OFFICE DE L'ENVIRONNEMENT DE LA CORSE-DEPARTMENT CONSERVATOIRE BOTANIQUE NATIONAL DE CORSE (OEC-CNBC)

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Xylella Fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy

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3RD JOINT ANNUAL MEETING

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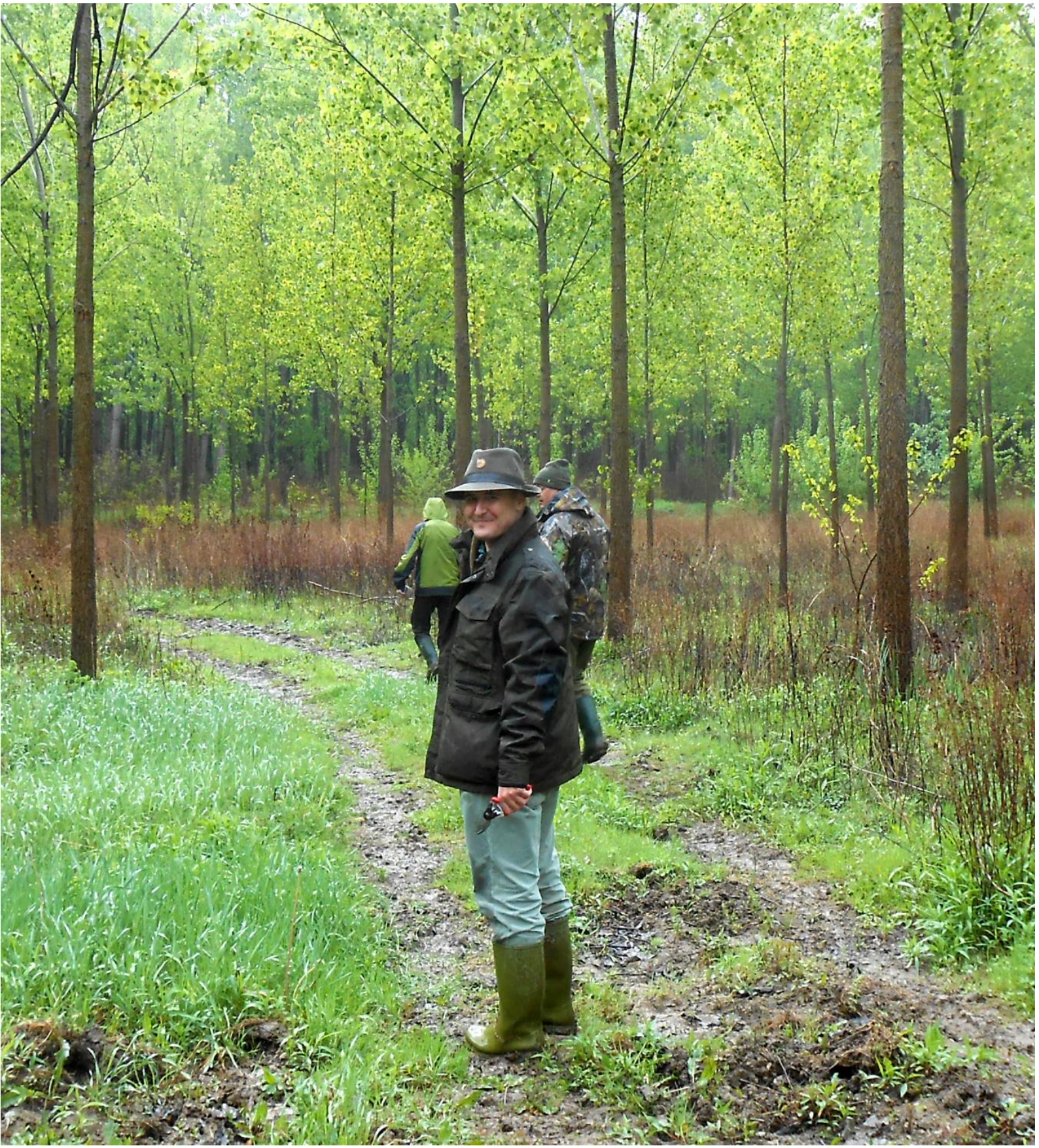

Obituary
Prof. Dr. Nenad Keča
1975–2019

Prof. Dr. Nenad Keča (https://www.ponteproject.eu/people/nenad-keca/) was born on October 25, 1975, in Senta, Serbia. He completed an elementary school in Kanjiža, and a high school in Senta. Prof. Dr. Keča received a BSc degree from the University of Belgrade, Faculty of Forestry, Department of Forestry, in 1999 with a grade point average of 9.72. Thereafter, he enrolled in postgraduate studies "Protection of Forests and Ornamental Plants" in 1999/2000 and defended his master thesis entitled "The study of the most important fungal diseases of *Populus* x *euramericana* Dode (Guinier) and the possibilities of suppression" in 2001. He received his PhD degree in 2005 by defending the doctoral dissertation titled "Biodiversity of *Armillaria* species and their role in the decay of trees in conifer and deciduous forests of Serbia and Montenegro" at the University of Belgrade, Faculty of Forestry.

After graduation, Prof. Dr. Keča worked as a forestry engineer in the Public Company "Srbijašume" for six months. Then, he joined the University of Belgrade, Faculty of Forestry as a teaching assistant. In 2016, he was elected full professor of forest and ornamental plant protection. At the age of 41, he became the youngest full professor in the history of the Faculty of Forestry. Prof. Dr. Keča was also involved in teaching Pathology of Forest Trees at the Faculty of Agriculture in East Sarajevo (forestry study program), in the period of 2014–2015. Teaching activities of Prof. Dr. Keča also included mentoring of students at all study levels.

During the period of 2003–2004, Prof. Dr. Keča visited the University of Aberdeen as a researcher in the laboratory of Prof. Dr. Steve Woodward, and was trained in forest pathology. Later, from May to September 2005, he stayed as a Norwegian Government Fellow at the Institute of Forestry (today NIBIO), Department of Forest Protection, with Prof. Dr. Halvor Solheim. During this period, he specialized in forest pathology. During 2018, he stayed for three months at the Institute of Forestry in Poland, specializing in root and tree sprout rot etiology.

Prof. Dr. Nenad Keča participated in the implementation of 20 projects (4 international and 16 national), and 5 COST Actions (FP0801, FP1002, FP1103, FP1203, FP1406), resulting in 104 papers. He made the greatest contribution in studying poplar pathogenic fungi, *Armillaria* spp., *Heterobasidion* spp., *Phytophthora* spp. and *Chalara fraxinea*. He detected some of those species for the first time in Serbia. He published more than 150 papers in international and national journals, and participated in numerous scientific meetings. Prof. Dr. Keča coauthored two university textbooks (Forest Phytopharmacy in 2010, and Forest Mycology in 2016). Prof. Dr. Keča was also a member of the Editorial Board of the following journals: Annals of Forest Research (SCI, Impact Factor 0.418), SEEFOR (South-East European Forestry) Regional Journal, Acta Scientiarum Polonorum, Silvarum Colendarum Ratio et Industria Lignaria. He was an editor-in-chief of the Faculty of Forestry Newsletter from 2009 to 2013, as well as an ad hoc reviewer in the following journals: Forest Pathology, European Journal of Plant Pathology, New Zealand Journal of Forest Science, Baltic Forestry, Annals of Forest Research, SEEFOR, Journal of the Faculty of Forestry and Papers of the Faculty of Forestry in Sarajevo.

The professional career of Prof. Dr. Keča is full of exceptional achievements thanks to his tremendous efforts, endless enthusiasm and devotion to his duties. He was a man of enormous energy, persistence and patience. Furthermore, he maintained high professional and personal standards serving as an example for his students and colleagues. By teaching and sharing his knowledge and expertise, he built an academic foundation out of 20 generations of students that will last for a long time. On August 21, 2019, his family lost a son, a husband and a father, and society lost a respected and distinguished scientist and professor.

Rest in peace, dear friend and colleague.

On behalf of the POnTE University of Belgrade team,
Prof. Dr. Aleksa Obradović
University of Belgrade, Serbia

MEETING PROCEEDINGS

Emerging pathogens of forestry

Oral presentation

Insights into the biogeography and global diversity of *Phytophthora*

Jung T*, Milenkovic I, Corcobado T, Tomšovský M, Janousek J, Panek M, Ďatková H, Balci Y, Scanu B, Brasier CM, Webber JF; Pérez-Sierra A, Bakonyi J, Seress D, Durán A, Tarigan M, Oliveira L, Sanfuentes von Stowasser E, Magnano di San Lio G, Schena L, Mosca S, Thu PQ, Nguyen Minh C, Maia C, Engelen A, Carella G, Moricca S, Cacciola SO, Pane A, La Spada F, Kageyama K, Hieno A, Masuya H, Uematsu S, Talgø V, Redondo M, Oliva J, Cravador A, Chang T-T, Fu CH, Horta Jung M

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Oral presentation

Phytophthora species in natural and seminatural ecosystems in Serbia; diversity, distribution and aggressiveness

Milenković I*, Keča N, Karadžić D, Tomšovský M, Radulović Z, Jung T

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Abstract: During the studies of Phytophthora species in Serbian ecosystems, different symptoms indicative for Phytophthora infections were recorded, such as increased crown transparency, yellowing of leaves, dieback of crowns and branches, collar rots and aerial bark cankers, root necrosis and extensive fine root losses. Some Phytophthora diseases were for the first time recorded in Serbia, like the declines of Picea omorika, Prunus laurocerasus or Juglans regia. Also, during the monitoring of forestry and ornamental nurseries in Serbia dieback, root rot, root loss and bark cankers of seedlings of Robinia pseudacacia, J. regia and Magnolia spp. were for the first time recorded in Serbian nurseries. Sampling and isolation were performed from forest stands, forest streams and rivers, nurseries and plantations, using standardized methods (Jung et al. 1996; Jung 2009). Identification of the obtained isolates was performed by observation of sexual and asexual structures under the light microscope and by sequencing of the ITS and other gene regions (White et al. 1990; Jung et al. 2017). The aggressiveness of selected Phytophthora isolates and species was tested under controlled conditions using standardized soil infestation and underbark stem inoculation tests (Jung et al. 1996; Milenković et al. 2018). In total, 46 streams and rivers were baited within 39 forest stands using 71 baiting rafts, and more than 700 Phytophthora isolates and many Pythium/Phytopythium-like isolates were obtained. In addition, 33 mixed soil samples from eight hosts were taken in 14 different forest stands and ca. 300 Phytophthora isolates were obtained. In addition, 29 soil samples were taken from 11 hosts in five nurseries, and more than 150 Phytophthora isolates were obtained. From forest streams and soils, nine different species were isolated, including P. gonapodyides, P. lacustris, P. chlamydospora, P. plurivora, P. ×cambivora, P. gallica, P. europaea and two undescribed taxa P. sp. organica and P. sp. Kelmania. From nurseries, the most frequent species were P. plurivora, P. ×cambivora and P. cactorum, but also P. gregata was isolated for the first time from Magnolia seedlings. In different pathogenicity tests, various Phytophthora species were aggressive to their respective hosts, like P. cactorum and P. sp. Kelmania to Picea omorika, or P. pini and P. plurivora to poplar plants in soil infestation tests; or P. plurivora to maple and poplar, P. ×cambivora to wild cherry and cherry laurel, or P. ×serendipita to wild pear plants in underbark stem inoculation trials, respectively.

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Oral presentation

POnTE Project: *Phytophthora* spp. on trees in Britain through the tree health diagnostic and Advisory Service at Forest Research

Pérez-Sierra A*, Gorton C, Lewis A, Chitty R, Van der Linde S, Armstrong A, Hendry S, Green S, Brasier C, Webber J

**Forest Research, Alice Holt Lodge, Farnham, England (UK)*

Abstract: Diseases caused by Phytophthora species have consistently been in the top ten reported problems on trees at the Tree Health Diagnostic and Advisory Service (THDAS) at Forest Research. A total of 227 Phytophthora reports have been submitted to THDAS since 2015 on 26 different tree genera. Phytophthora was isolated from these hosts and where possible was identified to species level by sequencing their ITS region with the primers TS4-ITS6. In 195 cases Phytophthora was identified to species level and in 32 cases confirmation was only based on the positive result of a lateral flow device (LFD). In total 19 different Phytophthora species were identified of which three were first records for Britain, P. foliorum on Rhododendron, P. siskiyouensis on Alnus incana and the hybrid P. gonapodyides x P. chlamydospora on Fagus sylvatica. The five most common identified species were P. plurivora followed by P. austrocedri, P. cinnamomi, P. pseudosyringae and P. cambivora. Phytophthora plurivora was recorded in 48 cases, 24 of which were identified on bleeding lesions on Tilia; P. austrocedri was mainly recorded on roots of Juniper; P. cinnamomi was mainly detected on roots/soil of Castanea sativa and P. pseudosyringae was primarily detected on bleeding lesions of Fagus and Nothofagus. Pathogenicity tests were carried out and Koch's Postulates were confirmed for the species that were detected for the first time in Britain. Furthermore, the susceptibility of different hosts to P. foliorum and of five different Alnus species to P. siskiyouensis was tested under laboratory conditions to assess their potential threat to British trees. Results on the species detected, the main hosts affected, and the value of the reports received at THDAS will be discussed.

Acknowledgement

The current staff at Forest Research would like to thank all the advisors that have contributed to THDAS records since 2015.

Oral presentation

Overview of *Hymenoscyphus fraxineus* in Britain through POnTE Project

Pérez-Sierra A*, Van der Linde S, Gorton C, Chitty R, Lewis A, Needham R, McCartan S

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Oral presentation

Monitoring of ash dieback in Austria and relations of *Phytophthora* species to decline of alpine green alder (*Alnus viridis*)

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Candidatus Liberibacter solanacearum and psyllid vectors

Oral presentation

Effect of temperature and inoculum load on *Candidatus* Liberibacter solanacearum disease symptoms and concentration in carrot plants

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Oral presentation

Vibrational communication and mating behaviour of the psyllid *Bactericera cockerelli*

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Abstract: An insect pest reliant on vibrational signals for mate finding can potentially be manipulated by means of mechanical stimuli, in order to both monitor and control the target species populations in the field. This strategy could be used to manage the tomato potato psyllid, Bactericera cockerelli, which is a major pest of various solanaceous plants. To assess the role of vibrational communication in pair formation, we therefore conducted a series of bioassays and recorded the emitted vibrations using a laser Doppler vibrometer from a leaf surface. On a potted bell pepper leaf, we released either individuals of a male (n=56) and a female (n=37) or pairs (n=62). A pre-recorded vibrational signal was transmitted to the leaf using a mini-shaker to evaluate whether the male (n=19) could be attracted towards the source point. We described the pair formation process and characterised both the male and female vibrational signals associated with mating. Mating signals were mostly initiated by the male (84%) and a vibrational duet was then established with the females that responded to their call (57%). During the duet, the male searched for the female, which remained stationary on the leaf. Mating was achieved (76%) if the male could continue to elicit the female reply and use her vibrations as cues to localise her. In a second experiment, the playback transmission of pre-recorded signals led most males to reach the source point, and to remain for a long period of time on the stimulated area, while fewer males of the silent control group walked towards the mini-shaker. We concluded that B. cockerelli uses species-specific vibrational signals to identify and localise a suitable mate. For this reason we hypothesize that the mating behaviour of this species is likely to be vulnerable to manipulation by means of vibrations. More research will be conducted to improve the attractiveness of the stimulus, in order to develop a mechanical monitoring and/or control technique.

Oral presentation

Developing of automatic devices mounted on a terrestrial vehicle for field monitoring of CaLsol and implementation of permanent surveillance system of psyllids

Blasco J*, Marco-Noales E, Fereres A, Alegre V, López S, Barbé S, Chueca P, Sanjuan S, Aguilar E, Navarro I, Ruiz C, Aleixos N, González-González G, Cubero S

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Abstract: A remote-controlled electric robot has been built to inspect the presence of CaLsol in horticultural crops by remote sensing. The devices used in the robot are three different DLSR cameras (one colour and other two modified for to capture NIR and BNDVI images), one thermal camera, one multispectral camera (eight bands between 550 and 850 nm) and one hyperspectral camera (400- 1000 nm). All cameras were placed facing the ground (plants). To prevent the influence of the uncontrolled natural light, the inspection area that was protected from outside light with a canvas and hence four halogen spotlights were used to light the scene. The images captured were georeferenced using a GNSS receiver (resolution of 3 cm). To guide the robot and synchronize the acquisition of images, a customized electronic board was developed using an inductive sensor coupled to the wheels of the robot. A custom software installed on an industrial computer allowed to control the acquisition of the images and the geolocation. During four seasons, monthly inspections have been carried out on several test plots of carrots in Villena (Alicante, Spain) trying to detect asymptomatic plants infected with CaLsol. Along the different seasons, the robot has received several improvements in order to increase its performance. This last year a new thermal camera has been used to solve the problems encountered in previous versions. Also, some errors have been detected and corrected in the geolocation software. Field maps with a high resolution (between 1 and 2.5 mm/pixel) have been created using vegetative indices from the spectral data. During the last trial, 50 plants were tagged to be identified in the images and collected separately to be analysed using real-time PCR. The robot has been effective in inspecting the fields and obtaining valuable remote sensing data. However, the predictive models created from the information obtained by the sensors did not allowed clear results for the early detection of the disease.

On the other hand, a prototype of surveillance station has been created to monitor potential vectors trapped in the field. The device captures and sends images of trapped insects, including potential vectors of CaLsol, to a remote server for the further inspection of an entomologist. The device was created on the basis of a Raspberry PI platform and incorporates a camera to capture the images, a control board to program the intervals of image capture and a modem to send the images and additional information to a remote server via 3G. The device can be programmed to capture and send the images at certain times of the day. A solar panel allows to power the devices for several weeks without the need of being recharged. The implementation has been carried out on Irwin type traps, consisting of a ceramic tile with a rough texture and a color and reflection spectrum similar to that of the leaves of the plant. The tile is placed horizontally inside a transparent methacrylate box at a height similar to that of the culture that is filled with a 50% solution of ethylene glycol water. The insects are attracted by the reflection of the tile and are trapped in the liquid. After several previous versions, the device have been fully redesigned and are being tested in the carrot test field in Villena. The devices have been programmed to send images of the insects trapped every three hours, and also the temperature of the field, to a remote server. In addition, a customised software has been developed to facilitate the analysis of the insects present in the images of the traps.

Oral presentation

Laboratory and field investigations into vertical transmission of CaLsol in parsnips, and practical application in seed production

Denton G*, Yao C, Preston J, Gawthrop F

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Abstract: Detection of Candidatus Liberibacter solanacearum (CaLsol) in Pastinaca sativa (parsnip) seeds and plants has impacted production and distribution of parsnips seeds. Vertical transmission of CaLsol has been investigated in Apiaceous crops but not within parsnip. Using the DNA extraction and real-time PCR protocol described by Bertolini et al (2014), including using propidium monoazide (PMA) to assess presence of living bacterium, parsnip seeds infected with CaLsol were identified and subsequently used to investigate vertical transmission. Laboratory experiments investigated detection of CaLsol from infected seed through early stages of growth (seed, imbibed seeds, radicles present, and cotyledons present). Preliminary results suggest detection at dry seeds stage only, with initiation of imbibing resulting in CaLsol either absent or below the detectable threshold of the real-time PCR. Covered polytunnel crops grown under insect proof mesh investigated movement of CaLsol from infected seeds to plants. Fifty plots of contaminated seeds were planted, along with 5 plots of uncontaminated seeds under insect proof netting. Leaf samples taken of the cotyledons, first true leaves and subsequently monthly were analyzed. Living or total CaLsol DNA via real-time PCR was not consistently detectable, with retesting producing negative results. Uncovered field trial of 140 plots that included 2 carrot varieties and 3 parsnip varieties were also undertaken. DNA extraction and real-time PCR was carried out on the cotyledons, first true leaves and after 11 months. Initial results detected CaLsol present, but retesting and verification of initial results with further dilution resulted in zero presence of CaLsol for all samples tested. Two trials looked at different disinfection treatments of parsnip seeds, in 2018 and 2019. During 2018, all treatments produced detectable levels of CaLsol. Whilst 2019 had shown possible reduction, eradication of living CaLsol was not effectively achieved. Testing of seeds for the presence of CaLsol has found certain requirements for production of CaLsol-free parsnip seeds in Europe. During 2017, 2018, and 2019 parsnip seeds (14, 13, and 13 samples respectively) were tested for the presence of CaLsol and only those produced under cover were found to be CaLsol-Free.

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Oral presentation

Temporal and biological dynamics of "*Candidatus* Liberibacter solanacearum" haplotypes D and E under natural conditions: data collection in carrot fields in Southwestern France

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Oral presentation

In silico approach for the design of a culture medium for `*Candidatus* Liberibacter solanacearum´

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Abstract: Until now, it has not been possible to achieve the growth of 'Ca. Liberibacter solanacearum' (CaLsol) in the laboratory. We are working in a genome annotation-based in silico approach for the design of a culture medium for this bacterium. The first consideration is to understand how this bacterium is provided with carbon and energy source compounds, as well as nitrogen and sulphur compounds. Thus, none of the sequences in its gene repertoire suggests that it is capable of transporting sucrose or fructose, leading to hypothesize that glucose is a major form of reduced carbon, and therefore glucose may be the main or the only source of energy and carbon. CaLsol can only synthesize de novo 6 aminoacids, and none of them is asparagine or tryptophan. Moreover, CaLsol lacks the enzymes required for incorporation of sulphur-containing inorganic compounds into amino acids, but the reduction of sulphate by a non-canonic enzymatic process or the use of an alternative terminal electron acceptor under anaerobic conditions cannot be ruled out. Also, it appears to be able to incorporate ammonia into glutamine. Concerning vitamins, no sequences matching complete transporters for riboflavin, pyridoxal phosphate, niacin, cobalamin, biotin or folate were found in the genome. Thiamine cannot be synthesized, but all three constituents of a typical prokaryotic thiamine ABC transporter are present. The source of NAD is nowadays unknown because it cannot synthesize or transport niacin. This bacterium lacks the Opp ABC oligopeptide transporters which partly accounts for the recycling of wall cell peptides, so the presence in the culture medium of cell wall components should aid in the growth of it. Culture temperature should not surpass 32.5ºC as cell membrane LpxL is absent in CaLsol. We are currently working on two approaches to try to achieve the growth of CaLsol in the laboratory, always taking into account some of the main traits of the genome of this bacterium. On the one hand, the use of complex culture media which, according to their general composition, could allow the growth of bacteria with significant deficiencies. On the other hand, the use of defined culture media supplemented with carrot phloem extract and other additives.

Oral presentation

Epidemidological study of '*Candidatus* Liberibacter solanacearum' in France

Loiseau M*, Gombert J, Le Roux AC, Sauvion N, Renaudin I, Forveille A, Coussy B, Morel E, Berton L, Villeneuve F, Ouvrard D, Samson-Kermarrec F, Laurent E, Poliakoff F

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Abstract: Haplotypes A and B of 'Candidatus Liberibacter solanacearum' (Lso) are associated with Zebra Chip of potato in USA. Following the first detection of Lso haplotypes D and E in France in 2012 in carrot crops and considering the potential economic impact of this bacterium for Apiaceous and Solanaceous productions, research programs (CASDAR French CaLiso and H2020 POnTE projects) have been implemented to further understand the epidemiological situation. In association with growers and researchers, data sheets and a survey plan were designed to collect plants and potential vectors. Surveys were planned in all areas with significant production of Apiaceous or Solanaceaous crops. For each surveyed field, the survey plan consisted in the collection of four symptomatic and two asymptomatic cultivated plants (five if no symptom occurred), four weeds of Apiaceous or Solanaceous genus and four plants at the edge. Whenever possible, psyllids have been collected. Plant collected samples and psyllids were analysed by real-time PCR to detect Lso. Morphological and molecular analysis were carried out to identify psyllid species. Surveys were implemented in 2016 and 2017 mainly on carrot seed crops and seed potato crops. Thirty-two French departments were surveyed corresponding to the main areas where carrot and potato seeds were produced in France. Despite the relative low presence of symptoms (approximately 90% of survey fields show between 1 and 10% of symptomatic plants or no symptom), our study reveals the presence of Lso in many carrot seed crops. Moreover, the psyllid Bactericera trigonica was caught in carrot seed crops and Lso was detected in 65% of the tested psyllids. However, the prevalence of Lso in carrot crops for consumption and industry is low, these types having a shorter life cycle than seed crops. For potato crops, no symptoms were observed. Furthermore, the psyllid B. cockerelli was not observed in France and Lso was not detected in potato samples. In conclusion, our large surveys show that even if Lso is present in carrot seed fields in France with its psyllid vector B. trigonica, the impact of this pathosystem appears as a minor problem in carrot crops. Additionally, the complex Lso-B. trigonica is not a problem in potato crops in France. The main risk for potato production would probably be the introduction of the psyllid vector, B. cockerelli.

Oral presentation

Does carrot seeds should be considered as a major pathway for transmission of '*Candidatus* Liberibacter solanacearum'

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**ANSES-LSV-UBVO Angers (FR)*

Abstract: 'Candidatus Liberibacter solanacearum' (Lso) is a bacterium associated with vegetative disorders in plants of Solanaceaous and Apiaceous families. Following the detection of Lso in carrot in Europe and particularly in seed carrot crops in France, in 2014, two experiments of seed transmission were conducted in two independent laboratories. The results of those laboratories were not the same: one team concluded that Lso could be transmitted by carrot seeds and the other never observed seed transmission. In order to dispel the hypothesis that these discrepancies are linked to differences in agronomic conditions, the trials were renewed in 2015 at Angers ANSES-LSV-UBVO. Four lots of 500 carrot seeds naturally contaminated with Lso and two lots of 100 healthy seeds were sown in an insectproof NS2 greenhouse. Sets of 108 plants from the contaminated lots and 24 plants from the healthy lots were individually analysed each month using real-time PCR to detect the bacterium. The detection tests on seeds and plants from healthy lots were always negative. During the 6 months of the trial, no plants from the contaminated seed lots tested positive for the bacterium or showed any infection symptoms. Since these studies, different teams around the world tried to reproduce the seed transmission but none of them manage to demonstrate it.

Oral presentation

Seasonal abundance of psyllid species associated with carrot and potato fields in Spain

Antolínez CA, Moreno A*, Ontiveros I, Pla S, Plaza M, Sanjuan S, Palomo JL, Sjölund MJ, Sumner-Kalkun J, Arnsdorf YM, Jeffries CJ, Ouvrard D, Fereres A

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Abstract: In recent years psyllids (Hemiptera: Psylloidea) have emerged as important pests in European agriculture because of their ability to transmit the phloem restricted bacterium 'Candidatus Liberibacter solanacearum' (Lso). In Europe, this bacterium causes severe losses to carrot and celery crops and represents a potential threat to the potato industry. The concern that Lso may be transmitted from carrot to potato and within potato has driven the need for monitoring populations of psyllid species that could serve as vectors on both crops, providing a fundamental understanding of the epidemiology of this bacterial pathogen. Different sampling methods were used to survey populations of psyllid species in commercial carrot and potato fields in Central and Eastern mainland Spain from 2015 to 2017. Two psyllid species, Bactericera trigonica and Bactericera nigricornis were found to be mainly associated with carrot and potato crops. In carrot fields the most abundant species was B. trigonica occurring from crop emergence to harvest, whereas in potato the most abundant psyllid species was B. nigricornis. Maximum psyllid numbers occurred between June and October its timing depending on the field location. Since B. nigricornis was found on both carrot and potato and is the only psyllid species able to feed and breed on both these crops in Europe, there is the potential risk of Lso transmission from carrot to potato. Valuable information was obtained to enable carrot and potato growers to design management practices to control psyllid populations and prevent Lso epidemics.

Oral presentation

'Candidatus Liberibacter solanacearum' haplotype c in Finland

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The access to the content of this abstract is restricted to registered members of POnTE Project. Please contact the corresponding author Anne Nissinen (anne.nissinen@luke.fi) for further information.

Oral presentation

Monitoring 'Candidatus Liberibacter solanacearum' (Lso) and its psyllid vectors across Europe

Sumner-Kalkun JC*, Arnsdorf YM, Back E, Carnegie M, Jeffries C, Sjolund MJ, Highet F, Ouvrard D, Greenslade AFC, Bell JR, Will T, Sigvald R and Kenyon D

**SASA, Edinburgh, Scotland (UK)*

Abstract: The phloem limited bacterium 'Candidatus Liberibacter solanacearum' (Lso) and its insect vectors are a major threat to the carrot and potato growing industry. Using suction trap networks across Europe and field scale sampling, this bacterium and its associated psyllid and plant hosts have been monitored to further understand the potential risk of Lso outbreak. In total 1483 psyllid specimens were screened for Lso from Germany and UK. 490 psyllids tested positive for Lso; including members outside the Triozidae family. The only psyllid found to be infected from suction trap samples from Germany was Trioza urticae which was carrying Lso haplotype U. Haplotype C was found in psyllids and plants within the UK along with novel Lso haplotypes from psyllids not previously reported to harbour Lso. Comparisons of suction trap screening and in-field sampling suggest that in-field sampling is the best way to monitor Lso and field scale psyllid diversity. To identify psyllid species a DNA barcoding database was built using ITS2 and CO1 gene regions from 60 psyllid species from 14 different countries. The psyllid DNA database was used to survey psyllid diversity and ecology and as a basis to design qPCR diagnostic assays to rapidly identify important psyllid vectors of Lso such as Bactericera cockerelli, B. nigricornis, B. trigonica, and Trioza apicalis. These will be important tools in the prevention and detection of outbreaks of psyllids such as Bactericera cockerelli which is currently an EPPO quarantine pest. Ongoing studies are concerned with assessing the impact of newly described Lso haplotypes and their psyllid hosts; and potential transmission to important crop plants.

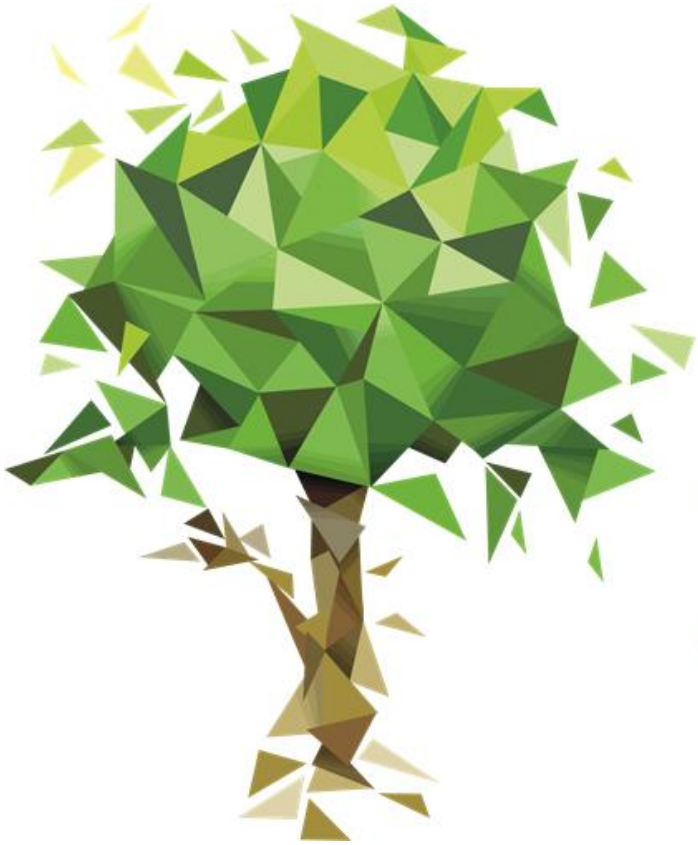
Oral presentation

Pest survey card on *Candidatus* Liberibacter solanacearum

European Food Safety Authority (EFSA), Loiseau M, Schrader G, Camilleri M, Diakaki M, Vos S*

European Food Safety Authority (EFSA), Animal and Plant Health Unit (ALPHA), Parma (IT)

Abstract: The European Commission requested EFSA to support the EU Member States in the planning and execution of their survey activities for quarantine plant pests. In particular, EFSA is asked to provide scientific and technical guidelines in the context of (i) the new plant health regime (Regulation (EU) 2016/2031), in which prevention and risk-targeting are given an extra focus, and, (ii) the European Commission co-financing programme of the annual Member State survey activities for pests of EU relevance (Regulation (EU) No 652/2014). EFSA is currently preparing a toolkit for risk-based surveillance, including practical pest survey cards for 52 quarantine pests. In the context of detection surveys and the related delimiting surveys implemented following a positive finding, the pest survey cards guide the reader through the relevant information and parameters needed for designing statistically and risk-based surveys. In particular, for Candidatus Liberibacter solanacearum, the information presented in the survey card includes: the pest's biology and ecology, its detection and identification and the key elements for survey design. This presentation describes the principles of a risk-based approach, and, the data requirements for performing a statistically sound sample size calculation, with Candidatus Liberibacter solanacearum as a case study.



2nd European conference on *Xylella fastidiosa* 2019

HOW RESEARCH CAN SUPPORT SOLUTIONS

Ajaccio, 29-30 October 2019

The abstracts collected in this session represent POnTE and XF-ACTORS contribution to the Second European conference on *Xylella fastidiosa*.

The conference was organised jointly by EFSA; the French National Institute for Agricultural Research (INRA); the French Agency for Food, Environmental and Occupational Health and Safety (ANSES); the Office de l'Environnement de la Corse (OEC) through its department the Conservatoire Botanique National de Corse; the EU-funded projects POnTE, XFACTORS, CURE-XF and EuroXanth; and the Euphresco network for phytosanitary research coordination and funding.

Xylella fastidiosa: Opening session

Oral presentation

Major results and challenges of the EU H2020 project POnTE on the control of *Xylella fastidiosa*

Boscia D*, Saponari M

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Abstract: 'Pest Organisms Threatening Europe' (POnTE) is a four-year H2020 project (grant agreement number 635646), started in November 2015, targeting emerging pests that threaten EU agriculture and forestry to develop knowledge and tools to minimise their impact and the risk of introduction in pest-free areas. The bacteria *Xylella fastidiosa* and *Candidatus* Liberibacter solanacearum and their vectors, as well as the phytopathogenic fungi causing major diseases in forestry and landscape trees, i.e *Phytopthora* spp. and *Hymenoscyphus* spp., were studied by a multidisciplinary team representing more than 20 partners.

The most relevant part of the project workplan was dedicated to studying the biological, genetic and epidemiological aspects of the bacterium *X. fastidiosa* spreading among olives in southern Italy.

The bacterium, its vectors and the host response was explored using innovative approaches and the knowledge gathered has significantly improved the current methods for disease surveillance and prevention. The preliminary results of the studies on the spittlebugs, olive microbiome and mechanisms of resistance in olive cultivars opened up new opportunities for the implementation of future applied research programmes on sustainable control strategies. The research programme has been supported by considerable communication and dissemination actions, promoting research networking, harmonisation of the surveillance strategies and transfer of knowledge to stakeholders. Scientific evidence collected as part of the project has been used to support and update the pest risk assessment and to implement the legislative measures enforced at EU and national levels.

The most significant achievements of the project will be briefly illustrated.

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Xylella fastidiosa Biology and pathogenicity

Oral presentation

Understanding the potential origin and epidemiological consequences of the Spanish outbreaks caused by*Xylella fastidiosa* subspecies multiplex

Landa BB*, Castillo Siri A, Giampetruzzi A, Román M, Velasco MP, Marco-Noales E, Moralejo E, Saponari M, Navas-Cortés JA, Almeida R

**Instituto de Agricultura Sostenible, Consejo Superior de Investigaciones Científicas (IAS-CSIC), Córdoba (ES)*

Abstract: Outbreaks in Europe associated with *Xylella fastidiosa* (Xf) subspecies multiplex are the most frequent, accounting for the larger number of susceptible plant species (more than 70%), with some host overlap among the different regions. Diverse sequence types (ST) have been detected across distinct geographical regions: ST6, ST7 and ST79 in Corsica and the Provence-Alpes-Côte d'Azur region (France), ST7 and ST81 in the Balearic Islands (Spain), ST6 in the province of Alicante and Madrid (mainland Spain), ST7 in the Douro Littoral region (Portugal), and ST87 in the region of Tuscany (northern Italy). In recent years, genetic analysis and pathogenicity tests have provided evidence of biological, ecological and host range diversity among strains of the same subspecies and STs. Draft genomes of 12 Spanish isolates of Xf subsp. multiplex (ST6 and ST81) were used for comparative genomic studies with currently available genomes of the same subspecies from France and Italy. Phylogenetic analysis based on core genomes, accessory genomes and single nucleotide polymorphisms indicate that: (i) European outbreaks associated with strains of Xf subsp. multiplex most likely result from distinct independent introductions; (ii) ST6-strains recovered from Spain and France, although sharing the same ST, fell in distinct phylogenetic subgroups; (iii) ST81 strains from the Balearic Islands and ST6 strains from Alicante differentiated in distinct phylogenetic groups (i.e. ST81 isolates are closer to ST6 isolates from California and France than ST6 isolates from Alicante, which in turn are closer to ST7 isolates from the USA and France); (iv) a low number of SNPs are detected among the strains recovered in Alicante, suggesting a recent introduction to the area. Additionally, whereas some recombination events were found among ST81 isolates from Mallorca and ST6 isolates from France with isolates of Xf subsp. fastidiosa ST1 from Mallorca, no evidence of recombination among ST6 isolates from Alicante with other European Xf isolates was found. Pathogenicity tests on the three main Spanish olive cultivars are being conducted with isolates belonging to ST6 and ST81 and compared with Xf subsp. pauca ST53 from Italy and ST80 from Ibiza. This work highlights that although the use of the MLST approach is a powerful tool for resolving genetic relationships among isolates, the exploration of the whole genomes provides more comprehensive information that in the future may help to retrieve more robust correlations with the biology and host range of the sequenced isolates. Furthermore, since current European regulation of Xf is based on the subspecies present in each outbreak, these results combined with further pathogenicity tests on the main crops may help to establish management and regulation policy standards for the affected areas in Europe.

Acknowledgements

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

Oral presentation

Evolutionary history of *Xylella fastidiosa* based on comparative genomics

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**IRHS, INRA, AGROCAMPUS-Ouest, Université d'Angers, Beaucouzé (FR)*

Abstract: Once thought to be restricted to the Americas, the plant-associated bacterium *Xylella fastidiosa* has been blooming since 2013 in Europe and Asia. This bacterium infects a wide host range and is responsible for plant diseases with great socioeconomic consequences. Genetically diverse, this species is divided into subspecies but genetic traits governing this classification are poorly understood. We used the comparative genomics of a set of nearly 50 genome sequences to gain better knowledge on the genetic traits associated with lineages and gene fluxes among lineages. We developed software, SkIf (specific k-mers identification), to mine genome sequences and identify signatures of groups of interest. Genome sequences were also used to gain knowledge of the evolutionary history of the subspecies multiplex. Altogether, we provide important resources and knowledge to optimise the strategies attempted to limit the pathogen dissemination in novel areas.

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Oral presentation

Insights into differential responses of olive cultivars to *Xylella fastidiosa* infections

Abou Kubaa R, Giampetruzzi A, Altamura G, Zicca S, Boscia D, Saponari M, Saldarelli P*

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

Abstract: *Xylella fastidiosa* strain De Donno causes severe symptoms of desiccation on the susceptible cultivars Ogliarola salentina and Cellina di Nardò. In the *Xylella*-ravaged olive groves, survivor plants of cv Leccino have been identified and monitored since the beginning of epidemic spread of *X. fastidiosa* in Apulia (southern Italy). Studies in field-grown plants (Giampetruzzi et al., 2016) suggest that the resistance of these two cultivars relies on two pillars: a lower bacteria population size compared with that of susceptible cultivars and, limited to Leccino, a differential gene expression response that involves leucine rich receptor-like kinases (LRR-RLKs). Successive studies with artificially infected olives under controlled conditions, showed that the same host responses occurred when plants of the cvs Cellina di Nardò and Leccino were inoculated with the strain CO33, taxonomically related to subsp. sandyi. Consistent with the previous data, transcripts of two LRR-RLKs, orthologous to At1g35710 and At4g08850, which are reported to regulate cell wall damage response in Arabidopsis thaliana (Van der Does et al., 2017), were found overexpressed in Leccino. Moreover, quantitative PCR assays targeting the At1g35710 olive orthologous gene showed an increased expression in different olive cultivars artificially inoculated under controlled conditions. Besides these molecular studies, the bacterial population sizes were estimated in different tissues of the infected plants: leaves, young (Ø?5 mm) and hardwood cuttings (Ø?5 mm -?1 cm), and in tissues collected from scions of different cultivars grafted onto the same rootstock. From these tests, further evidence on the resistance of Leccino and FS17 were collected, with tissues of these cvs harbouring lower bacterial titer than Ogliarola salentina (this was particularly evident in the leaves of the cv Leccino) and not showing typical and severe desiccation phenomena, even when branches of these cvs were co-grafted on the same rootstocks with scions of Ogliarola salentina showing severe dieback. Further data on these double grafted olives and genetic achievements will be presented.

Acknowledgement

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

Oral presentation

Can genome sequences tell us anything worthwhile about *Xylella fastidiosa* ecology?

Almeida R*, Castillo SiriA, Kahn A, Vanhove M, Sicard A

**University of California, Berkeley (US)*

Abstract: While the benefits of using *Xylella fastidiosa* whole-genome sequences for academic research are rather obvious, it remains to be shown that these data provide tangible information of immediate relevance to affected stakeholders, disease managers, and policy makers. More specifically, the question is whether whole-genome sequences provide additional and reliable information when compared with other approaches such as multilocus sequence typing and biological assays, for example. Using a data set of approximately 350 genome sequences, we have been asking questions on pathogen dispersal as well as what happens after introductions into new environments. We will provide examples and posit questions on the relative value of genome sequencing data to study and monitor *X. fastidiosa* ecology.

Oral presentation

Spatial distribution and genetic structure of *X. fastidiosa* subsp. *pauca* in olive trees in south-east Brazil

Safady NG*, Soares KC, Armange E, Silva LFO, Lopes JRS, Coletta-Filho HD

**Centro de Citricultura Sylvio Moreira -IAC / Universidade Federal de São Carlos (BR)*

Abstract: The bacteria *Xylella fastidiosa* (Xf) is considered to be a non-specific plant pathogen, infecting a hundred plant species and causing disease in dozens of them. Various subspecies are known, but the Xf subsp. *pauca*, Xf subsp. *multiplex*, and Xf subsp. *fastidiosa* are known to cause disease in economically important crops in the Americas and recently in Europe. In South America, Brazil, the subsp. *pauca* is known mainly to cause the citrus variegated chlorosis in sweet orange, but was also described as causing the olive quick decline syndrome disease, specifically in the south-east region. The total area occupied by olive trees in south-east Brazil is no more than 2000 ha which are located at high altitudes with small and dispersed orchards surrounded by native vegetation. We investigated, by PCR and isolation on BCYE medium, the geographic distribution of bacteria by analysing samples from 24 different orchards present in São Paulo and Minas Gerais States. Also, the population structure of 158 Xf isolates obtained from olive plants located in 10 different geographic regions was analysed by 12 single sequence repeats loci multiplexed in four sets for the PCR and electrophoresis. The forward primers were labelled with different dyes and the amplicons run by capillarity electrophoresis. Xf was detected in 20 out 24 sampled orchards (83%). Of a total 158 isolates, 64 different multilocus microsatellite genotypes were observed, i.e. 40%. Unbiased Nei's genetic diversity corrected by the population size (HNei) index ranged from 0.00 to 0.55 (average of 0.24 for all populations), lower than obtained by subsp. *pauca* populations from citrus and coffee (Francisco et al., 2017). FST index (Wright's fixation index) for all pairwise comparison of populations ranged from 0.075 ($p>0.01$) to 0.968 ($p<0.001$), meaning a strong subdivision among some populations, even with the recent outbreak. Discriminant analysis of principal components and PCoA via Nei unbiased genetic distance clustered the samples into three mainly genetic groups.

Support: Horizon 2020 (XF-Actors project number 727987) and FAPESP (São Paulo Research Foundation – projects number 2016/02176-7 and 2017/15092-9).

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Oral presentation

Phenotypic characterisation of two Spanish strains of *Xylella fastidiosa* subsp. *multiplex* ST6 differing in plasmid content

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**Instituto de Agricultura Sostenible, Consejo Superior de Investigaciones Científicas (IAS-CSIC), Córdoba (ES)*

Abstract: Two *Xylella fastidiosa* (Xf) subsp. *multiplex* strains IVIA5901 and ESVL were isolated from symptomatic almond trees in Alicante (Spain). Even if these Xf strains show an average nucleotide identity at the chromosomal level of 99.99%, they differ by the presence of two plasmids pXF64-Hb_ESVL and pUCLA-ESVL, only found in strain ESVL. Xf colonisation 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. The goal of this study was to characterise the phenotypic characteristics of these strains that may be related to their differences in plasmid content.

The Spanish Xf subsp. *multiplex* strains were compared with Xf subsp. *multiplex* strains Alma-Em3 and BB08-1 isolated from blueberry and the reference strain Xf subsp. *fastidiosa* Temecula1 from grapes, all isolated in the US. To study bacterial behaviour 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. Additionally, virulence assays were conducted in the greenhouse using tobacco plants cvs. SR1 and Xanthi. We also determined the presence of genes coding for type I and type IV pili in the Spanish strains. Our results show that the two Spanish isolates of Xf subsp. *multiplex* have lower motility, less capacity for aggregation, make less biofilm, and cause lower disease severity when compared with the US isolates used in this study. Also, there were significant differences in disease severity between Xf Spanish strains that varied according to the tobacco cultivar, with Xanthi being the most susceptible one. Besides, our results indicate that strain ESVL exhibits stronger attachment to substrate than IVIA5901, but no differences in biofilm formation, cell–cell aggregation or twitching motility were found between them. The role of plasmids in the biology of these Spanish Xf strains will be further investigated.

Study supported by project 727987 XF-ACTORS (EU-H2020), COST Action CA16107 EuroXanth and E-RTA2017-00004-C06-02 from AEI-INIA Spain and FEDER.

Oral presentation

Ancestral state reconstructions of *Xylella fastidiosa*–host plant relationships

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**University of California, Berkeley (US)*

Abstract: The broad host specificity that *X. fastidiosa* exhibits globally contrasts with the increased plant host specificity for individual strains. Understanding the molecular underpinnings of plant host specificity in *X. fastidiosa* is vital for predicting host shifts and epidemics. While there are multiple genetic determinants of host range in *X. fastidiosa*, there should still be detectable genomic evidence of the unique relationships between *X. fastidiosa* and its hosts. The objective of this project is to use phylogenetics to predict the ancestral plant hosts of *X. fastidiosa*. We used genomic data to construct phylogenetic trees of subsets of the core and accessory genomes at varied clade depths. With those trees, we created maximum likelihood ancestral state reconstructions of plant host at several taxonomic scales (species, genus, and multi-order clade). While some genomic regions were not historically informative in terms of predicting ancestral host state, others predicted high likelihoods of particular ancestral plant hosts at ancestral nodes. In future work, these same historically predictive genome regions could be used to identify genetic underpinnings of host specificity and be integrated into modelling potential host jumps and host range changes of individual strains of *X. fastidiosa*.

Poster

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

Sicard A*, Saponari M, Vanhove M, Giampetruzzi A, Loconsole G, Saldarelli P, Boscia D, Almeida RPP

**Department of Environmental Science, Policy, and Management, UC Berkeley, Berkeley, CA (US); UMR BGPI, INRA, Montpellier (FR)*

Abstract: The introduction of a *Xylella fastidiosa* (Xf) subsp. *pauca* strain in Italy has resulted in the first disease epidemic of this pathogen in Europe. We cultured the bacterium and performed whole-genome sequencing of over 70 Xf. subsp. *pauca* isolated from olive trees from 2013 to 2017 across affected areas in southern Italy. We identified several genes under positive selective pressure within the Italian population; these are genes that might be involved in the adaptation of *X. fastidiosa* to olive trees. Other aspects of epidemiological relevance, such as estimating the date of introduction of *X. fastidiosa* to Italy, are also being extracted from this dataset and will be presented.

Poster

Host plant range of different *Xylella fastidiosa* subspecies in experimental tests

Cesbron S*, Beaurepère Q, Sochard D, Denancé N, Marfisi S, Jacques M-A

**IRHS, Agrocampus-Ouest, INRA, University of Angers, Beaucouzé (FR)*

Abstract: *Xylella fastidiosa* (Xf) is an insect-transmitted bacterium, which infects the xylem of a wide range of plants. This plant pathogenic bacterium is genetically diverse with five proposed subspecies that have different regions of origin and were once thought to have different host ranges. Since the first detection of Xf in Europe in 2013, various strains of at least four subspecies were reported. The De Donno strain from the subsp. *pauca* infects mainly olive trees, but has been recovered from more than 20 other plant species. Strains from the subspecies *multiplex*, *sandyi*, *fastidiosa* and *pauca* have been identified in Spain, in France and in the north of Italy infecting more than 60 plant species altogether. Large overlaps in host range of the various subspecies were noticed. However, these host lists came from analysis of naturally occurring infections that depends largely on uncontrolled factors such as host availability where the strain is present and the presence of insect vectors attracted by these plants. The potential host range of the different strains circulating in Europe or dread strains remain poorly understood. We tested the pathogenicity of seven strains belonging to different subspecies (*fastidiosa*, *pauca*, *multiplex* and *sandyi*) on a large range of plants of interest for French horticulture such as citrus, grapevine, olive trees, plum and apricot trees. Survival at the inoculation point, colonisation at a distant point, and development of symptoms were quantified after pin-prick inoculation and incubation in confined growth chambers over 6 to 18 months. Generally, in these conditions symptoms were difficult to quantify and analyse, except on grapevine. Strain ability to disseminate in the plant from the inoculation point varied for a same plant species upon cultivars. Survival at the inoculation point was also a differential characteristic among strain–plant combinations. Our results will be useful for risk management in Europe.

Poster

Genomic analysis and biology of a novel variant of *Xylella fastidiosa* subspecies *multiplex* infecting different host plants in Tuscany, Italy

Saponari M*, Giampetruzzi A, Castillo Siri A, D' Attoma G, Altamura G, Saldarelli P, Almeida R, Boscia D

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

Abstract: An increasing number of *Xylella fastidiosa* outbreaks associated with strains of the subspecies *multiplex* has been reported in southern Europe, currently the most common strains being detected in Corsica, the Balearic Islands, the province of Alicante (Spain) and, more recently, reported for the first time in Portugal and in the region of Tuscany (northen Italy). The Tuscany outbreak raised major concerns, being associated with a new variant of *X. fastidiosa* subsp. *multiplex*, characterised as sequence type ST87, capable of infecting, among the others, almond. Although this sequence type is closely related to the more common ST6 and ST7 variants, remarkable differences were recorded when culturing the ST87 isolates on different media as well as when comparing their draft genomes. In contrast to the majority of the strains of the subspecies *multiplex*, the ST87 isolates grow on PD3 solid medium, a peculiar feature described for a few and highly virulent strains associated with almond leaf scorch disease in North America (Almeida and Purcell, 2003). Analyses based on core genome alignments and single nucleotide polymorphisms of currently available *Xylella* genomes show they are genetically related to strains previously characterised in North America, but in a separate clade from strains of the subspecies *multiplex* previously sequenced in Europe, supporting the hyphothesis that they originate from a distinct introduction that occured in Europe. Pathogenicity tests on *Prunus* spp. and grapes are ongoing to assess the virulence and the host range of this newly discovered variant of *X. fastidiosa*.

Acknowledgement

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

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Poster

Identification of multilocus SSR markers to assess the genetic diversity of *Xylella fastidiosa* subsp. *pauca*, ST53, spreading in Apulia (southern Italy)

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Abstract: The epidemic spread of *Xylella fastidiosa* in Apulia (southern Italy) with the continuous expansions of the border of the infected area, prompted several investigations into the genetics and genomics of the strains associated with the infections. These studies aimed to find genetic correlations with known strains of the bacterium as well as among the initially discovered Apulian outbreaks and those that emerged from time to time. MLST and full genome sequencing provided clear evidence that a single genotype, denoted ST53, of the subspecies *pauca* was causing the epidemics among olives and other hosts. In this work, we tested a panel of SSR markers in the attempt to disclose information on the genetic diversity and evolution of the bacterial population, even if, given the reduced spatial and temporal scales of this recent epidemic, a relatively low level of variation was expected. Five SSR markers selected among those previously reported in the literature proved to be polymorphic for the ST53-isolates from Apulia, that even if yielding a small number of alleles (from 2 to 5), when combined provide a good resolution, distinguishing several genotypes. Additionally, the design of a new set of 12 SSR markers for fine-scale genotyping of the Apulian isolates, yielded higher number of allelic variation, paving the way to perform micro-evolutionary and epidemiological studies. Indeed, the successful use of these markers on DNA plant samples, will allow a large-scale study, taking advantage of a six-year dataset of plant DNA collected and stored as part of the official monitoring programme started in the region at the beginning of the epidemics in 2013 and covering the entire demarcated area in Apulia that currently exceeds 200,000 ha.

Acknowledgement

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

Poster

Screening olive germplasm for resistance to Olive quick decline syndrome caused by *Xylella fastidiosa* under field and controlled conditions

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Abstract: Searching for resistance is regarded as one of the most promising long-term control strategies against the olive quick decline syndrome (OQDS) caused by *Xylella fastidiosa* (Xf). A set of 60 olive genotypes are now being screened for resistance to OQDS under field and controlled conditions. Plants consisted of self-rooted plants, produced at IFAPA in Córdoba (Spain) and then transferred to the CNR-IPSP-Bari (Italy). A first batch of 10 genotypes from different *Olea europaea* subspecies and 10 breeding selections from the IFAPA breeding programme was planted in an open field under high disease pressure in November 2016 and inoculated under controlled greenhouse conditions in March 2017. A second batch of 40 cultivars selected from the World Olive Germplasm Collection, representative of the genetic and geographical variability of the collection, was inoculated under controlled greenhouse conditions and exposed to natural infections in summer 2017. Preliminary results indicate differences in the incidences of the infections and, among those that support systemic infections, differences were recorded for the presence and severity of shoot dieback and desiccation phenomena. Results gathered under controlled conditions made it possible to identify some genotypes in which poor systemic infections (only a few replicates were colonised) were detected even upon two rounds of inoculations. Conversely, symptoms of shoot dieback were recorded on some accessions, most probably indicating they are particularly sensitive to the infections. Interestingly, the selection of Leccino confirmed previous evidence, with inoculated plants harbouring low bacterial population size and not showing severe shoot dieback. However, especially for the field experiments affected by the weather and climatic conditions, observations and quantitative assays need to be prolonged in order to acquire conclusive data from multi-year surveys. On the basis of these preliminary results, a first set of progenies from tentative resistant genitors are being currently developed for future studies.

Poster

Lack of evidence for seed transmission of *Xylella fastidiosa* subsp. *pauca* from infected olive trees and annual host plants

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Abstract: In 2013, *Xylella fastidiosa* emerged in southern Italy threatening mainly olive trees, which upon bacterial infections succumb to severe desiccation and rapid decline. High rates of infected and symptomatic trees are usually recorded in the contaminated olive groves. Such evidence prompted several investigations to assess the pathways of local spread of the infections. Beside graft-/insect vector-mediated transmission, the possibility that the pathogen may be vertically transmitted through infested seeds was also investigated, by testing seeds collected from naturally infected olives and weeds (*Erigeron* spp. and *Chenopodium album*).

Four lots of olive fruits were harvested in January 2014 and 2016 from infected olive trees selected in three different locations in the Apulia region (southern Italy). Seeds were cleaned from the pulp and used either for the diagnostic tests (qPCR assays) or stratified at 4°C for three months followed by germination. For diagnostic tests, 24 seeds for each source were used to test either the excised embryos or the endosperm plus the seed coats.

Upon germination, the number of seedlings recovered varied between 30 and 50 for each lot, with a total of 160 seedlings grown in confined conditions for five years. Diagnostic tests on seedlings were performed one year after the germination and then repeated three (seeds collected in 2016) or five years (seeds collected in 2014) later.

Similarly, for the infected weeds diagnostic tests were performed (i) on groups of seeds (>100 seeds/sample) harvested in 2016 from infected plants, and (ii) on six-month-old plantlets obtained after seed germination.

The results of the qPCR assays on the seeds and on the recovered seedlings (both for olives and weeds) unequivocally indicated lack of positive detections, supporting the evidence of lack of seed-to-seedling transmission of this bacterium as previously shown for other susceptible crops.

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Poster

Studies to elucidate the cause of alteration in colony morphotype of *Xylella fastidiosa* subsp. *pauca*, ST53

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Abstract: Isolation in pure culture of *Xylella fastidiosa* (Xf) strains from infected olives showing severe decline in southern Italy, represented a major breakthrough for advancing the research on this first and most severe bacterial outbreak which emerged a few years ago in Europe. However, during an extensive isolation campaign, some of the cultured isolates (Xf subspecies *pauca*, ST53) started to show an odd shape and growth pattern. Briefly, after an initial growth, the colonies started to become translucent, of reduced size, roughness and adherent to the media. Passage of these isolates on new agar-plates, regardless of the media, evolved in cell death. Microscope examination using the BacLight LIVE/DEAD bacterial viability staining kit and observations at the Transmission Electron Microscopy (TEM) revealed that the odd-shaped bacterial colonies consisted of dead cells, and the presence of different bacteriophage-(like) particles were observed in the TEM preparations. Ultrathin sections of these bacterial colonies showed a higher number of intracellular vesicles/endospores than those observed in the cells with regular morphotype, and their content discharged into the extracellular space. Filtrated suspensions prepared from altered colonies scraped from the plates, were able to reproduce similar alterations when mixed with ST53-isolates (subsp. *pauca*) of the regular morphotype. Conversely, no effects were noticed when strains belonging to other subspecies were put in contact with the same filtrates.

Studies are in progress to understand the nature of these intracellular vesicles/endospores which resemble a sort of 'pseudolysogenic phages' (Ripp S and Miller RV, 1997) induced in other bacterial species as a strategy to enhance survival in unfavourable environmental conditions. Indeed, attempts to purify phage particles from cultures displaying odd colonies are ongoing to verify whether they originate by spontaneous induction from temperate phages, known to occur in the genomes of the ST53-isolates causing the infections in the epidemic area of Apulia (southern Italy).

Acknowledgement

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Poster

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

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Abstract: Understanding the mechanisms underlying the development of symptoms of olive quick disease syndrome (OQDS) and the different olive cultivar responses to infections caused by *Xylella fastidiosa* subsp. *pauca*, ST53, is fundamental for disease control. The combined action of bacterial aggregates and plant-derived vascular occlusions (tyloses, gums and gels) has been invoked as the cause of the pathogenic alterations occurring in *Xylella*-infected plants. Conflicting observations were found about the role of vascular occlusion in the disease progression in field-grown olives. In the present work the distribution of vascular occlusions in the secondary xylem vessels of susceptible, Cellina di Nardò, and resistant, Leccino and FS17, cultivars were studied by light microscope observations of toluidine blue stained sections, recovered from stems of greenhouse-grown mock and artificially inoculated olives. One-year-old shoot portions collected from symptomatic or symptomless twigs were firstly tested by qPCR and used to recover thin sections (0.2 mm thick), with a similar number of vessels inspected for each cultivar. In the sections recovered from the non-infected controls 0.15%, 0.02% and 0.13% of occluded vessels were present, respectively in Cellina, Leccino and FS17. These percentages increased in the infected twigs reaching 9.65%, 6.81% and 1.33% in Cellina, Leccino and FS17, respectively, indicating that *Xylella* infections (regardlessof the cultivar) induced occlusions of the xylem vessels; these were significantly higher in the susceptible cultivar. More specifically, percentages of occluded vessels in the susceptible cultivar ranged from 1% to 34%, while those of the resistant Leccino and FS17 ranged from 0.044% to 14% and 1.09% to 1.53%, respectively. Although a clear-cut difference was observed among the infected cultivars, within each cultivar no significant differences were recorded between symptomatic and non-symptomatic shoots, suggesting that the development of symptoms is enhanced by a combination of different factors (anatomical, chemical and physical).

Poster

Antibiotic susceptibility and virulence profiling of endemic *X. fastidiosa* subsp. *fastidiosa* isolates from Costa Rica

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Abstract: *Xylella fastidiosa* subsp. *fatidiosa* is endemic in Costa Rica. Although the bacterium has great potential for disease and it is widespread throughout the country, a common feature is that infected host plants tend to be asymptomatic or in the worst case scenarios show mild symptoms as reported for 'crespera disease' in coffee. Another trait within this population is that isolates have shown broad genetic diversity, based on different typing techniques including MLST. Our recent efforts have focused on phenotypic characterisation of isolated strains. We tested antibiotic susceptibility of *X. fastidiosa* subsp. *fastidiosa* isolates to tetracycline, streptomycin, gentamicin, chloramphenicol, ciprofloxacin and penicillin. minimal inhibition concentration (MIC) (µg/ml) were generated using Etest® assay, a quantitative technique that has been used previously in slow-growing bacteria including *X. fastidiosa*, with reproducible results; a difficult task to achieve using other susceptibility determination techniques. Results show an overall susceptibility of the isolates to most of the antibiotic tested; however, differences in MICs within isolates for each antibiotic should not be overlooked. To evaluate whether the endemic *X. fastidiosa* subsp. *fastidiosa* population is capable of infecting and causing severe disease in *Nerium oleander* as seen for *X. fastidiosa* subsp. *pauca* (ST53), also present in Costa Rica, several isolates of *X. fastidiosa* subsp. *fastidiosa* representing different ST types, were assayed in a virulence test using *N. oleander*. Eight months after inoculation none of the tested isolates have induced evident symptoms of disease and bacteria were barely detected near the inoculation site six months post-inoculation. Monitoring is still ongoing.

This research was financially supported by funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 635646: POnTE (Pest Organisms Threatening Europe) and grant agreement No 727987: XF-ACTORS (*Xylella fastidiosa* Active Containment Through a multidisciplinary-oriented research strategy).

Poster

Leaf ionome profile of susceptible and resistant olive cultivars infected by *Xylella fastidiosa*

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Abstract: *Xylella fastidiosa* (Xf) is continuing to emerge as a devastating bacterial pathogen for many economically relevant species. Xf subsp. *pauca* strain De Donno is associated with olive quick decline syndrome (OQDS), a destructive disease occurring in the southern area of Apulia (Italy). In susceptible olive cultivars, symptoms are characterised by initial leaf scorch and scattered desiccation of small branches that over time worsen and extend to the whole canopy. Greenhouse and field observations revealed that olive trees of the cultivar Leccino show milder symptoms, when compared to those observed in Ogliarola salentina. Ogliarola salentina have progressive, severe scorching and complete dieback. Moreover, the lower bacterial population size in Leccino confirmed the resistance of this cultivar to Xf infection, suggesting that it is able to limit pathogen multiplication.

To understand the role that mineral nutrition may play in host resistance to OQDS, a field survey of the leaf ionome was carried out among trees of two orchards located in the Xf-infected demarcated area, that showed clear differences in response to Xf infection. Infected leaf samples, classified as symptomatic and asymptomatic, were subject to the determination of the ion content by inductively coupled plasma – optical emission spectrometry (ICP-OES). Data were analysed in relation to the different cultivars and the presence or absence of symptoms.

The comparison between symptomatic and asymptomatic samples showed an increase of sodium levels in both cultivars and significantly higher calcium levels in the symptomatic tissues of Leccino, a response that had been found in other Xf-host pathosystems. Otherwise, Leccino trees had a significantly higher content of manganese, in both symptomatic and asymptomatic leaf tissues.

These field observations inform currently ongoing experiments under controlled conditions to investigate the relevance of these mineral ion changes in the development and progression of symptoms, and the potential involvement of manganese in resistance of the Leccino cultivar to Xf infection.

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Poster

Monitoring of biofilm production in *Xylella fastidiosa* strain De Donno via biochemical signalling modulation

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Abstract: Diffusible lipid species are exploited by bacteria for regulating cell motility, cell-to-cell communication, activation of metabolism and proliferation. The most widely investigated lipid family is represented by diffusible signal factors (DSFs) responsible for quorum sensing. *Xylella fastidiosa* (Xf) uses the DSFs to coordinate genes involved in the expression of virulence and biofilm formation. These moieties are mainly cis-2-unsaturated fatty acids which directly enhance xylem infection and biofilm production, which are the two processes involved in the genesis of severe plant diseases such as the noticeable olive quick decline syndrome, associated with the Xf subsp. *pauca* strain De Donno and affecting olives in the Apulia Region. Here we report the results of studies aiming to identify DSF molecules of Xf De Donno and exploit strategies for modulating its biofilm formation.

We started inducing DSF expression in *Escherichia coli* using a plasmid vector recombinant for the *rpfF* gene of Xf De Donno and verifying the production of exogenous proteins and fatty acids. We set up extraction, mechanical treatments, and methyl ester derivatisation of the extracted crude oils from Xf and *E. coli* cultures. We compared the GC-MS profiles of fatty acids belonging to the metabolic activity of bacteria harbouring the bare and *rpfF*-recombinant plasmids. Building on previous studies, we speculated on the production of unsaturated fatty acids with a chain length of 12?18 carbon atoms, with?-unsaturated functions. Isolated and treated crude extracted oils obtained from the same bacterial sources, were tested *in vitro* to investigate their phenotypic effect on biofilm growth and the expression of key genes related to surface adhesion, biofilm formation and cell movement.

Furthermore, we set the synthesis of new, no commercially available, cis-2-unsaturated fatty acids with a chemical structure related to the DSF family, in order to test the *in vitro* alteration of biofilm production in Xf De Donno. The exploited reaction was the stereoselective Still-Gennari olefination which leads to the synthesis of unsaturated fatty acids in cis (Z) conformation starting from commercial aldehydes.

Results of these activities will be presented.

Poster

Experimental confirmation that *Xylella fastidiosa* subsp. *pauca*, ST53, does not colonise grapes

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Abstract: *Xylella fastidiosa* is able to colonise a very large number of plant species, but considering each subspecies/phylogenetic clade the number of associated susceptible hosts is significantly reduced. Although strains genetically related most likely share similar host range, using phylogenetic relationships to infer information regarding the potential host range of new strains is still problematic, and pathogenicity tests remain the only means to assess the capability of a given strain to infect or not a specific plant species. Based on the current European legislative provisions on *X. fastidiosa*, information on the host range of the strain(s) causing an outbreak have regulatory consequences. In this context, we have made efforts to prove experimentally the capability of *X. fastidiosa* subsp. *pauca*, ST53, one of the most virulent European genotypes, to infect grapes, using 23 grape varieties (*Vitis vinifera*) and four rootstocks. Upon needle inoculation, plants were monitored for 18 months, using standand diagnostic methods (qPCR and isolation), supported by vector-transmission tests and observation of thin sections of the inoculated stems, stained using the LIVE/DEAD BacLight kit. qPCR assays on samples collected at the inoculation points (i.p.) and from the distal portions, 6 and 12 months post-inoculation, yielded positive reactions in more than 90% of the i.p., whereas in half of the cultivars scattered amplifications (the majority yielding Cq values > 30) occurred in some replicates at 15–20 cm from the i.p., but none of the apical portions tested positive. Isolations made 18 months after the inoculation, either from mature leaf petioles and stems portions harbouring the inoculation points, failed to recover actively growing colonies. At the same time, microscope observation of the thin sections showed only the presence of aggregates of dead *Xylella*-cells at the i.p. Transmission tests performed using specimens of *Philaenus spumarius* caged on the inoculated grapes produced negative results for both insects and recipient plants. The overall results showed that the bacterium was successfully delivered into the stem of the grapes and bacterial residual could be qPCR-detected even one year after the inoculation, but none of the inoculated cultivars sustained active bacterial multiplication and colonisation.

Acknowledgement

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Poster

Transformation of *Xylella fastidiosa* subspecies *pauca* strain De Donno

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Abstract: *Xylella fastidiosa* subsp. *pauca* strain De Donno has been recently identified as the causal agent of a severe disease affecting olive trees in a wide area of the Apulia Region (Italy). While insights on the genetics and epidemiology of this virulent strain have been gained, the complex network of interactions with the main susceptible host remains to be explored. A fundamental tool for understanding such interactions is the development of bacterial mutants for functional analysis of genes involved in the host recognition, pathogenicity and insect transmission. Experimental studies have demonstrated the natural competence of *X. fastidiosa* in the uptake of exogenous genetic material; a feature exploited for site-specific introduction or deletion of genes through homologous recombination. Nevertheless, numerous studies have shown that several factors may affect *X. fastidiosa* transformation efficiency, including growth rate, twitching motility, sequence similarity, and the presence of restriction–modification systems that cleave incoming DNA. On this basis, two different plasmids containing the chromosomal replication origin (oriC) of *X. fastidiosa* and *E. coli* were used to transform *X. fastidiosa* De Donno in order to produce a GFP-expressing and a knockout strain for the *rpfF* gene, a crotonase producing a diffusible signal factor (DSF), involved in the quorum-sensing system. Repeated attempts to exploit natural competence, introducing the donor plasmids into *X. fastidiosa* De Donno failed, highlighting the critical role of genetic diversity in recombination performances of this pathogen. Conversely, GFP and RpfF mutants were successfully obtained by co-electroporation in the presence of an inhibitor of the Type I R-M system, that had been proved to impact the stable acquisition of foreign DNA by *X. fastidiosa* subsp. *fastidiosa*. Availability of mutants for one of the most virulent strains of *X. fastidiosa* opens for new explorations of host–microbe interactions, important to elucidate mechanisms underpinning the differential responses recorded upon infections of different olive cultivars and toward the implementation of strategies to mitigate the impact of the disease.

Acknowledgement

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Poster

Occurrence of plasmids pXF64-Hb_ESVL and pUCLA-ESVL associated with infections caused by *Xylella fastidiosa* subsp. *multiplex* ST6 in the demarcated area of Alicante, Spain

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Abstract: An outbreak of *Xylella fastidiosa* (Xf) subsp. *multiplex* ST6 was first identified in June 2017 in Alicante province (Valencian Community, Spain) affecting mainly almond trees, but also other species including ornamentals, cultivated *Prunus* spp. and landscape plants. The current demarcated area (DA) in Alicante covers 134,581 hectares, and affects more than 70 municipalities. Genetic analysis may provide evidence of biological, ecological and host-range diversity among Xf strains of the same subspecies and STs and help to elucidate the pathway of introduction and spread in an affected area. Pairwise comparisons of the chromosomal genomes of two ST6-sequenced strains isolated from almond trees in Alicante, ESVL and IVIA5901, showed an average nucleotide identity higher than 99.9%. Interestingly, the two strains differ for the presence of the plasmids pXF64-Hb_ESVL and pUCLA-ESVL detected only in the ESVL strain. The aim of this study was to determine the incidence and distribution of plasmid-bearing strains of Xf subsp. *multiplex* ST6 in Alicante. PCR tests were performed on 20 strains isolated from different hosts, and on approximately 100 DNA samples from infected almond trees collected in eight municipalities within the DA of Alicante. PCR results on the cultured isolates showed the occurrence of ST6-strains harbouring the two plasmids, or only the plasmid pUCLA-ESVL or none of them. Interestingly, in some of the DNA samples from infected almonds, only the plasmid pXF64-Hb_ESVL could be detected. More specifically, 4% of ST6-infected samples harboured the plasmid pXF64-Hb_ESVL, 12% only pUCLA-ESVL, 16% both plasmids, and 44% neither of the two plasmids. In five of the eight municipalities, we found samples harbouring the two plasmids, and only in the samples from the municipality of Benifato the PCR tests failed to amplify the targeted plasmids. Future work will increase the number of samples to cover the entire DA to better understand and relate the presence of plasmids to the epidemiology of this disease in combination with the use of multilocus sequence typing and genome sequencing of more Xf subsp. *multiplex* ST6 strains.

Poster

Characterisation of olive xylem microbiome community composition by metabarcoding greatly depends on the matrix used to extract DNA and 16S universal bacterial PCR primers

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Abstract: Understanding of xylem sap microbiome is becoming of relevant importance for plant health as it could include microbes that may protect against xylem-limited pathogens, such as *Xylella fastidiosa*, and supporting key biological processes. Furthermore, the negative pressure, low oxygen and nutrient content of the xylem sap make it a unique and unexplored microbial environment. In this study, we evaluated the differences obtained in the characterisation of the xylem microbiome composition when using xylem sap extracted from xylem vessels using a Scholander pressure chamber or when using macerated fine chips obtained from xylem tissues from 10-year-old or 1-year-old olive trees. We also compared four different PCR primer pairs targeting 16S rRNA for their efficacy to avoid co-amplification of mitochondria and chloroplast 16S rRNA, as this is an important drawback in metabarcoding studies. PCR primers tested included 799F/1062 (V5-V6), 799F/1115 (V5-V6), 967/1391 (V6-V8) and 799F/1193 (V5-V7). Illumina paired-end sequence quality control and chimeric filtering was performed with DADA2 using QIIME2. Taxonomy affiliation into OTUs at 99% was based on the Silva reference database. The highest mitochondria and chloroplast amplification was obtained when using xylem chips and 799F/1062 (77.7%) and 967/1391 (99.6%) primers. On the contrary, 799F/1115 and 799F/1193 primers showed the lowest mitochondria (< 6.76%) and chloroplasts (< 0.02%) amplification, and the highest number of OTUs identified, 245 and 247, respectively. Interestingly, only 81/236 and 27/240 OTUs or 66/144 and 21/149 genera were shared between xylem sap or wood shavings after amplification with 799F/1115 or 799F/1193, respectively. The most abundant bacterial genera (> 50% of reads) included *Anoxybacillus*, *Cutibacterium*, *Methylobacterium*, *Pseudomonas*, *Rathayibacter*, *Sphingomonas* and *Spirosoma*. However, their relative importance varied depending of the matrix and primer pairs used. These results will help to optimise analysis of xylem microbiome community composition and more importantly to understand its driving and modifying factors.

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Xylella fastidiosa: Detection and surveillance

Oral presentation

Novel and high-throughput diagnostic procedures to detect *Xylella fastidiosa* in plants and vectors developed within the POnTE project

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Abstract: The research activities of the H2020 project POnTE partners explored serological and molecular methods to detect *Xylella fastidiosa* (Xf). Their efforts were devoted to optimise and standardise the procedures for sample preparation and purification of DNA, which are critical because of the presence of inhibitors in many host plants. Laboratories implemented internal comparative studies for accuracy, specificity, sensitivity and detection limit evaluation. Along with interlaboratory comparison tests, they enabled the performance criteria of the diagnostic methods to be assessed. They make it possible to provide new set of data crucial to the revision of the EPPO Protocol (PM 7/24 (3)) and official methods. Direct sampling in the field based on prints on cards/membrane procedures for serological tests with the FTA-ELISA system as well as DTBIA are good alternatives to the standard sample extraction for ELISA. The Harper et al., (2010) protocol combined with the CTAB or DNA extraction kit is still the most accurate method on almond, olive and other host DNA extracts. Quick biomolecular tests usable in the field overcoming the preparation of sap and DNA extraction, such as olive prints on Whatman followed by qPCR, Lamp (Enbiotech kit) and RPA (Agdia kit) used with portable devices are promising in terms of performance criteria but there is still a lack of experience. The direct nucleic acid hybridisation on nitrocellulose strip (lateral flow) is also an alternative that provided an optimisation to avoid amplicon contamination being carried out. Detection of Xf in heads of *Philaenus spumarium* in pools of 5 to 15 insects is reliable as performance criteria are not altered compared with the analysis of individual heads of insect using Taqman PCR Harper et al., (2010) in Duplex with IOOS et al., (2009) whatever DNA extraction used. Rapid protocols for subspecies assignation (single nucleotide primer extension – SnuPE) and HMR combined with end-point PCR have shown promising results: the SNuPE method using a multiplex amplification of the *gyrB* gene to differentiate all subspecies and genotypes within Xf subsp. *Pauca* and a rapid and reliable protocol of HRM-analysis based on new set of primers in the *leuA* and *gyrB* genes are being evaluated.

Oral presentation

New tetraplex qPCR assays for simultaneous detection and identification of *Xylella fastidiosa* subspecies in plant tissues

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Abstract: *Xylella fastidiosa* (Xf) is an insect-borne bacterium confined to the xylem vessels of plants. This pathogen has a broad host range estimated to more than 560 plant species. Five subspecies of the pathogen with different but overlapping host ranges have been described, but only three are widely accepted. Detected in Europe since 2013, the management and regulation of its outbreaks in Europe depend on the subspecies. It is of major interest to identify it as accurately and as early as possible after infection. To improve Xf detection, three tetraplex qPCR tests were developed to identify the subspecies directly from plant material in a single reaction. All tests included primers designed to be specific to Xf species and different sets of primers targeting three subspecies. We designed primers and probes using SkIf, a bioinformatics tool, based on k-mers to detect specific signatures of the species and subspecies from a dataset of 47 genome sequences of Xf. We tested the qPCR assays on 39 target and 30 non-target strains, on samples of 13 different plant species spiked with Xf strains of different subspecies, and on naturally infected host plant samples. The primers and probes designed in this study were *in silico* as well as *in vitro* species- and subspecies-specific. On DNA, the sensitivity of single assays was equal or slightly better than the reference protocol, depending on the primers; and the tetraplex assays had the same sensitivity as the reference protocol. Tetraplex assays allow Xf detection up to 103 cells mL^{-1} in all tested matrices. On naturally infected samples, the tetraplex qPCR tests allowed subspecies to be identified at levels where MLST failed. Moreover, mixed infections of two to three subspecies could be detected in the same sample with the tetraplex assays. These qPCR tests are modular tools that are reliable and efficient for differentiating Xf subspecies directly in plant samples.

Oral presentation

A quick and efficient method for detection of *X. fastidiosa* in olive plants based on tissue-print

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Abstract: The commercial olive crop in southeast Brazil is relatively young, with plants no more than 20 years old. The olive orchards are located in a chain of mountains with altitude ranging from 800 to 1900 m, 1565 mm annual rainfall, and an average temperature of 19.4 °C, which results in biotic and abiotic disorders easily confounded with the olive quick decline syndrome (OQDS) symptoms associated with *X. fastidiosa* (Xf). The geographic localisation of orchards also makes it difficult for the shipment of samples (leaves) under conditions for diagnosis of Xf in the lab. Aiming to help the growers on identification of OQDS symptoms in suspicious plants by the correlation with positive diagnosis of Xf, we are optimising the serologic-based tissue-print (T-P) protocol on nitrocellulose membrane for detection of the pathogen. Polyclonal antibodies were produced in rabbits using whole cells of a mixture of Xf subsp. *pauca* strains. Twigs and roots with 3 to 6 mm diameters of plants with OQDS-like symptoms were squeezed and the sap blotted onto membrane. The result of the detection was visualised directly on the membrane through colour reaction peroxidase-biotin-conjugated antibody. The validation of the method was checked by qPCR and PCR. Of total samples with OQDS-like symptoms, in 90% of the cases, the presence of Xf was confirmed by T-P, in 88% by qpcr, and in only 33% by standard PCR. Xf was confirmed in 100% of the suspicious plants taking together both T-P and qPCR results. No infection of Xf was observed in healthy (asymptomatic) plants by the three tested methodologies. In conclusion, the T-P protocol was an efficient, cheaper and quicker methodology for Xf detection in plants with OQDS-like symptoms. Nitrocellulose membranes have been provided to the growers, which make the print of suspicious samples and later mail them to the lab. Interesting results have been obtained using this strategy. Support: Horizon 2020 (XF-Actors project number 727987) and FAPESP (São Paulo Research Foundation – project number 2016/02176-7).

Oral presentation

Optimisation of sampling and testing procedures for detecting *Xylella fastidiosa* in large lots of plant for planting and nursery stocks

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Abstract: Inspections and diagnostic tests for *Xylella fastidiosa* are mandatory on consignments/place of production for the most susceptible species listed in the EU Decision 2017/2352, as well as for the movement of 'specified plants' produced in nurseries located in the so-called demarcated areas. Although inspections and sampling are carried out in accordance with the ISPM31 standards, at laboratory level such requirements imply the manipulation of a large amount of material (leaves/shoots/cuttings). Attempts have been made to experimentally optimise a protocol suitable for the concentration of the bacterial cells starting from large volume of plant homogenate, prior to being tested with the protocols commonly adopted for detecting the presence of *X. fastidiosa* in plants. Diagnostic sensitivity of serological and molecular tests was assessed by using infected plant materials (*X. fastidiosa* subspecies *pauca*, ST53) spiked at different ratios with non-infected plant materials. Portions (single leaves or pieces of stems) of infected plants were individually tested to establish the distribution of the bacterium in a given infected symptomatic/asymptomatic plant. Briefly, petioles and/or stem portions recovered from infected plants of *Polygala myrtifolia*, *Nerium oleander*, *Olea europaea*, *Lavandula stoechas*, and scraped xylem tissue from infected cuttings of *Prunus avium* were pooled at different ratios with healthy materials up to 40 g/sample. Indeed, tests included plantlets from the species of the Brassicaceae and Solanaceae families (non-host plants of the ST53 strains) upon spiking the pooled samples with stem portions of infected periwinkle. After grinding the pooled samples, plant homogenates were centrifugated and the recovered pellets resuspended in the appropriate extraction buffer and processed using the standard serological or molecular protocols. The data gathered for sampling (minimum number of portions/aliquot) and for testing (maximum size of the pool) provide useful guidance when processing a large number of samples, satisfying sample size requirements while keeping the diagnostic tests technically and economically affordable.

Acknowledgement

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Oral presentation

Targeting surveillance for *Xylella fastidiosa* in Europe: an epidemiological basis

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Abstract: The emergence of *Xylella fastidiosa* in Europe has highlighted the importance of surveillance for protection of host plants against exotic pathogens. However, the surveillance strategy will depend upon the surveillance aim – whether this is declaring pathogen absence, preventing pathogen establishment, containing and delimiting new pathogen incursions, or mitigating pathogen impact. Our previous work has also demonstrated that the biological characteristics of the pathosystem and the performance and costs of the methods used to detect the pathogen must be considered when planning and evaluating surveillance. We can achieve this by linking epidemiological models of pathogen spread (to predict where and when disease will occur) with statistically-informed sampling models, and by using mathematical or computational strategies to interrogate the output. We describe here how to implement surveillance in areas where *X. fastidiosa* is thought to be absent, including the 'uninfected zone' of Apulia, Italy, and the United Kingdom. Surveillance in these areas must be implemented for long durations of time, and therefore must be efficient and sustainable. It is well recognised that surveillance costs can be reduced while maintaining an acceptable probability of pathogen detection by precise targeting of surveillance efforts, but the question remains as to how exactly to achieve this. For example, how should surveillance resources be balanced between sampling hosts and sampling vectors? Where should surveillance resources be placed in a large and heterogeneous landscape or trade network? And which detection methods should be used to detect the pathogen? We demonstrate how these questions can be answered using a variety of different spatial and non-spatial approaches. As well as providing specific recommendations for improving surveillance activities for *X. fastidiosa*, these methods are generic and thus can improve our understanding of surveillance systems in general for a range of pests and pathogens.

Oral presentation

Spatio-temporal monitoring of *Xylella fastidiosa* in olive trees using radiative transfer models and Sentinel-2 images

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Abstract: Detecting and monitoring the spatial and temporal dynamics of the symptoms and the severity of the damage caused by *Xylella fastidiosa* (Xf) is a key priority to prevent its expansion. This study evaluates the use of Sentinel-2 imagery together with a radiative transfer (RT) approach to monitor epidemics caused by Xf in olive trees. A time series of Sentinel-2a imagery collected over two years was used to describe the temporal dynamics of Xf-infected olive orchards located in the region of Apulia (southern Italy). Airborne hyperspectral acquisitions were used for validation along with field visual surveys carried out for more than three thousand trees with different disease incidence (DI) and severity (DS) levels. A careful evaluation of the sensitivity of Sentinel-2 imagery to canopy alterations produced by a progressive Xf infection in olive orchards has been accomplished based on model simulations and field observations.

Our results demonstrate that the assessment of Xf infection monitoring based on Sentinel-2 data requires the use of self-corrected vegetation indices (VIs) and RT modelling. Among the tested VIs, those that minimise the atmospheric and background effects such as ARVI, ATSAVI and OSAVI performed better than traditional vegetation indices used as a quantitative proxy measure of the fractional cover (FC) of green and healthy vegetation such as NDVI, RDVI or MSR. Model simulations and field observations showed that the background effects have a significant impact on the temporal variation of DI levels detected with Sentinel-2a imagery. The use of 3-D RT modelling improved the DI estimates by 25% when accounting for the background effects, and by 32% when its heterogeneity was also considered. Therefore, the methodology proposed using a 3-D RT and Sentinel-2 data can provide useful spatiotemporal indicators to track the damage caused by Xf infections across large areas.

Oral presentation

Optimisation of the delimiting survey strategies for *Xylella fastidiosa* in the demarcated area in Alicante

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Abstract: *Xylella fastidiosa* (Xf) is a regulated quarantine plant pathogen in the EU. The current legal provisions specify the implementation of delimiting surveys in infested areas to demarcate the geographic extent and implement eradication or containment measures. North-eastern Alicante Province, Spain, is one of the areas infested by Xf in the EU, and so it is currently demarcated and subject to a delimiting survey activity. Based on the 2018 official surveillance programme, in which approximately 100,000 ha were surveyed and 11,000 samples were taken and analysed, this work aimed: i) to estimate the spatial variation in disease prevalence; and ii) to improve the efficiency of the current delimiting survey plan.

Prevalence was modelled by means of a Bayesian spatial hierarchical model in which climatic and spatial factors were evaluated using INLA approximation. A delimiting strategy was designed based on a three-phase adaptive approach in which the surveyed area (epidemiological unit) size and sampling intensity were tailored according to the previous phase information. An algorithm was implemented to optimise the number of epidemiological units to be surveyed and the sample size by simulating different random sampling scenarios from the reference data. The strategy was evaluated by comparing the delimitation efficacy and prevalence estimates between the proposal and the reference data. Prevalence results revealed that the spatial component had a strong effect on disease spatial variation. The proposed delimiting strategy was able todelimit the disease to a similar extent (similar efficacy) with a lower number of samples (better efficiency) than the current one.

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Poster

Estimating the asymptomatic period of *Xylella fastidiosa* from incomplete data

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Abstract: Visual detection remains the primary frontline method of detection of *Xylella fastidiosa* infection in susceptible host plants. Surveillance systems for detecting and delimiting new incursions and for declaring pathogen absence from areas considered free of the pathogen are therefore impacted by the duration of the 'asymptomatic period' between infection and the first emergence of disease symptoms. However, relatively few data are available on the asymptomatic period of *X. fastidiosa*, especially considering the wide variety of bacterial subspecies and strains in different host plants and climatic conditions. Following a request from the European Commission as part of the recent EFSA update of the 2015 Xylella Pest Risk Assessment, we developed a novel strategy for estimating the duration of the asymptomatic period from data routinely recorded during experimental infection studies. Using the timing and numbers of symptomatic and asymptomatic hosts at first symptom development and the end of the study, we are able to estimate the probability of a given host not having developed symptoms at any given timepoint following infection. We do this using two methods: one assuming that symptom development occurs at a fixed rate over time (allowing us to extrapolate beyond the available data); and another which makes no such assumption (and thus more accurately represents the data available). Using data obtained from a comprehensive review of the literature on experimental inoculation studies, we found good agreement between both methods. Although care should be taken when interpreting the results, we are able to draw useful conclusions regarding the duration of the asymptomatic period for different *X. fastidiosa* subspecies in different hosts. Importantly, we find that almond infected with the *multiplex* subspecies, and sweet orange or olive infected with the *pauca* subspecies, remained asymptomatic for the longest durations (up to five years) after infection, with implications for ongoing surveillance activities.

Poster

Improvement of the sampling method for the monitoring of *Xylella fastidiosa* in Apulian olive groves

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Abstract: An effective monitoring programme for *Xylella fastidiosa* relies on accurate sampling procedures in the pathogen-free areas of olive trees, as the primary host of the bacterium, to detect the infection early before symptoms develop. In this study, the current method of sampling plant material for *Xylella* was improved in two olive groves (one of Ogliarola and one of Leccino cvs) located in the northern part of the infected area of Apulia. Sampling was carried out on olive trees with no or low degree of symptomatology, investigating the spatial and quantitative distribution of the pathogen at canopy level throughout the year (from December 2017 to March 2019). After assessing the total infection rate in the first sampling, 10 trees per grove were selected based on the absence or presence of mild symptoms (8 ELISA-positive and 2 ELISA-negative) for a more in-depth and accurate sampling at two canopy levels (low and high), which was conducted at 6 times: December 2017 (T0), March 2018 (T1), June 2018 (T2), September 2018 (T3), December 2018 (T4) and March 2019 (T5). During the sampling periods, temperature ranges and averages were also recorded by a climatic station in the area. Two matrices, twigs and mature leaves were used for the analysis. The same portion of the two matrices was analysed by serological methods (ELISA) and qPCR as single or composite samples. The number of positive samples from the high level of the olive canopy throughout the period was significantly higher than those from the low level, regardless of the cvs. However, a significant increase in the detection of the pathogen in the lower part of the trees was found in the period June–September only in the cv Leccino. In the same period, however, the results of the qPCR showed a decrease in bacterial content. As for the matrices, the twigs were the best compared with the lower portion of the leaf with petiole. The results on the spatial and quantitative detection of *Xylella* in the canopy and in the two matrices for (T4) and T(5) is underway as the correlation between the detection of pathogens and temperatures (averages and ranges). In addition, the sampling schemes at grove level are also under evaluation.

Poster

Specific PCR detection of *Pseudophaeomoniella* spp. in the xylem of healthy and *Xylella fastidiosa*-infected olive trees

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Abstract: Several fungal species have been found associated with the olive quick decline syndrome (OQDS) caused by *Xylella fastidiosa* subsp. *pauca* (Xfp) in Apulia, southern Italy. The xylem-inhabiting species, *Pseudophaeomoniella oleae* and *Ps. oleicola*, were associated with brown or black wood streaking of various olive varieties, both on young and centenarian trees. However, specific pathogenicity tests conducted on olive plantlets indicated that these fungal species have a marginal role in the aetiology of OQDS. Considering the wide distribution of *Ps. oleae* and *Ps. oleicola* over the olive-growing areas in Apulia, a genomic characterisation study was started, in order to investigate the biology of these fungi, and to ascertain any possible interaction with Xfp and the OQDS. Several PCR primers were designed from the internal transcribed spacer (ITS) regions of the rDNA genes of *Pseudophaeomoniella* spp. in order to develop a species-specific detection method. Primers were screened against the two reference strains, *Ps. oleae* FV84 and *Ps. oleicola* M24, and six more *Pseudophaeomoniella* spp. isolates, resulting in the amplification of a single specific amplicon from most of the primer pairs tested. Fifteen primer pairs confirmed their specificity when tested against several isolates of different xylem-inhabiting fungal genera, such as *Phaeoacremonium*, *Pleurostomophora*, *Phaeomoniella*, *Ochroconis*, *Paraconiothydium*, *Aspergillus*, *Lophiostoma* and *Cladosporium* spp. The size of the obtained amplicons enabled the use of some primer pairs in a real-time PCR test, using a SYBR® Green format. Results confirmed the specificity of the tested primers, thus allowing the detection and quantification of *Ps. oleae* and *Ps. oleicola*, into the xylem of both healthy and Xfp-infected olive trees. Further research is in progress to develop a specific probe for qPCR quantification of the targeted fungal species in the olive plant.

Poster

A combined analytical and hyperspectral approach for early detection of *Xylella fastidiosa* in olive plants: preliminary results

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Abstract: Among the several approaches to study the infection by *Xylella fastidiosa* (Xf) in olive plants, the combination of nuclear magnetic resonance (NMR), high resolution mass spectrometry (HRMS) and hyperspectral reflectance (HR) techniques is very promising as it provides a comprehensive view of the metabolome and the spectral range of infected olive plants. Indeed, NMR gives information on molecules with relatively high concentration (typically, primary metabolites), HRMS provides details on molecules at low concentration levels (typically, secondary metabolites) whereas HR offers valuable information on the biochemical and biophysical characteristics of the plant. Comparison of the metabolic and hyperspectral profiles of both infected and control plants can be exploited to identify markers of the infection and correlated wavelengths to set up analytical and hyperspectral strategies for early detection.

In this study our attention was devoted to the effects of infections by *Xylella fastidiosa* (ST53) in combination or not with fungi isolates correlated with the olive quick decline syndrome on olive leaf composition. Different sets of infected olive leaves were submitted to investigation also taking into account simultaneous infections by fungi isolates of *Phaeoacremonium* (F1 (*Rubrigenum*) + F2 (*Aleophilum*)) and *Pseudophaeomoniella* (F3 (*oleae*) + F4 (*oleicola*) + F5 (*oleicola*)).

NMR, HRMS and HR data of control and infected leaves were submitted to principal component analysis (PCA) to perform the following comparisons: Xf vs control; Xf vs (XfF1 and XfF2); Xf vs (XfF3, XfF4 and XfF5). NMR, HRMS and HR results indicated that, even though fungal infections are operative, *X. fastidiosa* causes peculiar changes of the metabolic and spectral profiles. In fact, Xf-infected samples are differentiated from those not infected by Xf. Metabolites mainly related to the infections by *X. fastidiosa* are Oleuropein derivatives.

The study is ongoing on the correlation of HR with HRMS and NMR specific statistical analysis.

Poster

A new device for rapid and on-site pathogen detection

Spoto G*

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Abstract: A prototype of a diagnostic device for molecular analysis has been designed, developed and realised.The device is able to fulfil every step needed to perform isothermal gene amplification: DNA extraction, amplification and real-time revelation through fluorescence analysis. The excitation and emission wavelengths are respectively 470 nm and 530 nm. With this device it's possible to perform 48 tests at the same time using standard 0.2 ml PCR tubes; specifically four different analyses can be performed at the same time, in 12 test groups each. The analysis duration depends on the specific analysis to be performed; it is generally between 30 and 60 minutes. To trace all performed experiments, a technology for operator and experiment identification has been integrated in the system; all information is recorded locally and in the cloud, allowing remote access through a safe and encrypted system. The extraction and amplification temperatures handled by the device are between 55 and 90 °C, with 0.1 °C accuracy. The device handling interface has been realised with an Android application on a tablet connected to the device via bluetooth; through the interface it's possible to:

- automatically identify the user, through the use of RFID badges/bracelets
- recognise the test type, through the use of microchips embedded in the kits
- recognise the sample that has to be analysed, through the use of printed or RFID labels or through the import of pre-loaded data from the server
- visualise real-time plots with the results of the analysis and experiment reports with data interpretation.

The device has been designed to have a small size to allow on-site direct use, through an (external) battery or through standard 12 V car plugs.

Poster

Comparison of real-time PCR protocols for detection of *Xylella fastidiosa* in different plant species and cultivars

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Abstract: Detection is one of the first steps to prevent the introduction and spread of *Xylella fastidiosa*. This task can be influenced by the bacterial concentration and the type of plant species analysed. The diagnostic standard for *X. fastidiosa* EPPO 2018 PM 7/24 includes several protocols of real-time PCR addressed to different genome targets. In order to determine the accuracy of these PCR protocols, samples of healthy plant material from five cultivars of almond (Marcona, Marta, Avijor, Belona, Constantí) and eight of olive trees (Alfafara, Arbequina, Blanqueta, Cornicabra, Frantoio, Hojiblanca, Manzanilla, Picual) were spiked with tenfold serial dilutions of the strain IVIA 5901 of *X. fastidiosa* subsp. *multiplex* ST6 isolated in the outbreak in Alicante (Spain). Spiked samples of other plant species such as grapevine, citrus, oleander, pistachio, loquat and persimmon are also being challenged. In all cases, the CTAB method was used for DNA extraction. Sensitivity is being analysed between PCR protocols for the same cultivar, as well as between species or cultivars for the same real-time PCR, and so far slight differences have been found. Moreover, these protocols are being assayed with samples of almond trees from the infected area in Alicante. In some cases, problems of inhibition have been detected with certain real-time PCR protocols. All these results can give clues to optimise the detection protocol depending on the host and the specific situation of the area of origin of the plant material analysed.

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Poster

Current situation after the first outbreak of *Xylella fastidiosa* in an olive grove in mainland Spain

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Abstract: A first case of infection caused by *Xylella fastidiosa* subsp. *multiplex* ST6 was detected in an olive tree in the municipality of Villarejo de Salvanés, in the southeast of the Community of Madrid (Spain) in April 2018. This constituted the first detection of *X. fastidiosa* in this crop on the Iberian Peninsula, and the second concerning other crops after the detection of the pathogen in almond trees in Alicante, in the Valencian Community (Spain), where *X. fastidiosa* subsp. *multiplex* ST6 was also identified, although until now it has not been detected causing infection in olive trees in that region.

The Community of Madrid accounts for 27,000 hectares of olive trees, most of them in Villarejo de Salvanés. This area has a continental climate, with below zero minimum winter temperatures. After the official declaration of the outbreak, the actions established in the European Decision 2017/2352, the Contingency Plan against *X. fastidiosa* of the Spanish Ministry of Agriculture, Fisheries and Food, and that of the Community of Madrid were immediately applied to eradicate the bacterium and prevent its spread.

Among these measures, around 2,000 samples from olive trees and other host plants of the demarcated area (DA), 300 insect vectors, and olive trees from other municipalities were analysed. All of them gave a negative result by real-time PCR official protocols. As a direct consequence of the application of the eradication measures, the results obtained so far would indicate that this detection was a unique event and the bacterium would be neither disseminated nor established in the EU, although surveillance measures within the DA must continue as established in the Action Plan of the Community of Madrid.

Poster

Detection and identification of *Xylella fastidiosa* in France: improvement of the detection scheme

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Abstract: *Xylella fastidiosa* was identified in France in 2015, first on Corsica, then in the Provence-Alpes-Côte d'Azur administrative region (French Riviera). Forty-nine host plants have so far been identified, infected by *X. fastidiosa* subsp. *multiplex* belonging to the sequence type ST6 or ST7. Also in these two areas, the vector *Philaenus spumarius* has been found positive for these two sequence types.

In France, the detection scheme for plants and vectors is based on the real-time PCR Harper et al. (2010) after a high-throughput DNA extraction based on the QuickPick™ Plant DNA kit used with a KingFisher™ robot. This method was evaluated in 2014–2015, showing excellent performance criteria, apart from a lesser sensibility on olive tree (*Olea europaea*) and some oak species (*Quercus* spp.). In order to improve the limit of detection on these matrices, collaborative works with INRA Angers led to the evaluation of a new protocol based on sample sonication, in order to break *X. fastidiosa* biofilms, CTAB DNA extraction and modified parameters for the real-time PCR mix. Although this method is time consuming, it allows a real improvement of the limit of detection for olive tree and holm oak despite the presence of PCR inhibitors.

Strain identification is performed using an MLST scheme (www.pubmlst.org) with the PCR Yuan et al. (2010) for amplifying sequences of seven housekeeping genes. On plant DNA extracts giving high Ct values with the real-time PCR Harper et al. (2010), some amplification failures were observed. A protocol with the addition of BSA into the PCR reaction mixture has been validated, showing improved performance in term of sensibility allowing success for strain typing even in the presence of a small amount of target DNA.

Poster

Different approaches for detection of *Xylella fastidiosa* by molecular techniques

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Abstract: The outbreak of *Xylella fastidiosa* in several European countries on many plant hosts make it necessary to have fast, sensitive, and specific methods that allow large-scale surveys in wide areas, in order to know the degree of dispersion of the pathogen. For this purpose, molecular techniques that do or don't require DNA purification have been developed in diagnostic kits by various companies. In this work, a comparative study has been made of some of these kits by analysing a set of infected almond tree samples from the demarcated area of the outbreak in Alicante (mainland Spain). The real-time PCR by Harper et al. (2010, erratum 2013) and Francis et al. (2006) after manual DNA purification by CTAB was considered the gold standard protocol. First, four different master mixes for real-time PCR were tested, and one of them was selected. Then, the methodologies to be challenged were the automatised DNA extraction with Maxwell® RSC PureFood GMO and Authentication Kit in a Maxwell® RSC instrument (Promega), the Xylella Screen Glow kit based on LAMP technology with the ICGENE system (Enbiotech), the AmplifyRP® XRT+ Isothermal Amplification kit based on recombinase polymerase amplification (Agdia) and phyAlert® kit based on a triplex PCR (MICROGAIA BIOTECH). Interestingly, DNA measurements were not informative of the sensitivity of the protocols challenged.

Similar qualitative results were obtained with all the protocols, each of them with advantages and disadvantages. The choice can be based on the sample size and the economic and human resources of the laboratory. In general, a test with no DNA purification could be used in infected zones for a first screening, further analysing the negative samples with DNA purification before real-time PCRs recommended in EPPO 2018 PM 7/24.

Poster

Supporting early detection of *Xylella fastidiosa* by using 'indicator plants' and improved molecular detections assays

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Abstract: Early detection of *Xylella fastidiosa* (Xf) in new areas constitutes a factor of fundamental importance for a successful eradication strategy of this pathogen. To this end, in the context of the European project 'XF-ACTORS', the Laboratory of Bacteriology of Benaki Phytopathological Institute (LB-BPI) is participating in a research task of this project to evaluate the use of selected plant species, exhibiting rapidly characteristic symptoms upon Xf infection, as 'indicator plants' in risky locations to support early detection of Xf. In this connection, LB-BPI is using such indicator plants exposed in selected high-risk areas in Greece, i.e. two harbours, an airport and an open market where high-volume trade of plants for planting occurs. The indicator plants have been placed in pots in the selected locations and are monitored and tested regularly according to EPPO-recommended methods (conventional PCR and qPCR). So far, none of the indicator plants has been found infected by Xf. Additionally, research is being carried out at LB-BPI to improve the sensitivity of the PCR-based methods by treating the plant DNA extracts before amplification with a commercially available mixture of polymeric materials to segregate PCR inhibitors. Moreover, in a parallel project of the LB-BPI, the possible interference in the Xf detection assays of the microbiome associated with Greek olive varieties is being examined to obtain relevant data to further support optimisation of the respective diagnostic protocols.

Poster

XylAppEU_2.1.3 for precise acquisition and traceability of monitoring data of *Xylella fastidiosa* in the EU

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**Centre International des Hautes Etudes Agronomiques Méditerranéennes – Mediterranean Agronomic Institute of Bari, Bari (IT)*

Abstract: XylAppEU 2.1.3 will be launched at the end of 2019 as the final version of the Android application XylApp_1.2.4 used for the official monitoring of *Xylella fastidiosa* in Apulia, Italy. It was developed on the basis of the characteristics and input received from stakeholders in three EU pilot areas (Córdoba, Spain; Crete, Greece; Apulia, Italy) and from other project partners. A video tutorial of the previous version (2.1.2) was prepared and launched on the project website and the application download was available for testing between partners. This version considers the following aspects which will be implemented with additional input from partners after testing: (i) analysis of the scenario in the specific country; (ii) identification of the specific needs and resolution conditions in relation to the different users; (iii) definition of the featured applications, constraints, performance, interfaces and any other features to fulfil users' needs; (iv) active engagement of end-users for the finalisation and validation of the app.

The advanced application XylAppEU_2.1.3 was equipped with grids of different sizes (e.g. 100 m x 100 m, 1 km x 1 km) of the three pilot sites, based on the free formats available from the European Environment Agency. With regard to the hardware and other minor functions, different parameters (sensor configuration, connectivity and size of the display) were implemented for the most popular platforms (e.g. Google Android). The following specific features were also implemented: transmission protocols for data sending over the internet; inclusion of grid visualisation of the pilot sites for sample localisation at EU level (ETRS89-LAEA Europe); development of a GIS model for management of EU cartographic data. A new graphic interface has been made, user friendly, which guarantees better functionality and integration of modules, beside guidelines or better management by users. This version allows the acquisition, storage and transmission of data in different formats. As for the previous version, a video tutorial on XylAppEU_2.1.3 will be prepared for dissemination purposes.

Poster

Harmonisation of laboratory diagnosis of *Xylella fastidiosa* among national reference laboratories

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Abstract: On 27 March 2019, the European Commission designated the European Union Reference Laboratory (EURL) for Bacteria in Plants that will operate within the framework of official controls in plant health. The EURL for Bacteria in Plants is a consortium consisting of four partners, namely: 1. the Research Centre for Plant Protection and Certification CREA-DC (Italy); 2. the Research Institute for Agriculture, Fisheries and Food ILVO (Belgium); 3. the National Institute of Biology NIB (Slovenia); and 4. the National Reference Centre NRC-NVWA (the Netherlands). The major objective of the EURL for Bacteria in Plants is to support the activities of the Commission in relation to risk assessment and risk management of plant pests, to facilitate and harmonise laboratory diagnosis and analyses and to coordinate activities of the corresponding national reference laboratories (NRLs).

The EURL for Bacteria in Plants aims to achieve an overall high level of diagnostics at NRLs. Among the organisms listed as Union quarantine pests, emphasis on the priority pest *Xylella fastidiosa* has been given for the period 2019–2020. Based on the consolidated Work Programme of the EURL for Bacteria in Plants, details and guidance on internationally recognised (e.g. EPPO diagnostic standard PM 7/24) test protocols for the detection of *X. fastidiosa* will become available to all NRLs. A PT will be organised in 2019 to assess the diagnostic competence of laboratories to detect *X. fastidiosa* in selected host plants. A questionnaire, prior to the PT, will collect information on the technical aspects of test protocols currently used by the NRLs.

Poster

Comparison of real-time PCR and droplet digital PCR for the detection of *Xylella fastidiosa* in plants

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Abstract: *Xylella fastidiosa* (Xf) is a quarantine plant pathogen bacterium originating from the Americas and that has emerged in Europe in 2013. Limited to the xylem vessels of plants, Xf has a broad host range estimated to encompass more than 560 plant species. Xf can be detected directly on plant macerate using molecular methods such as real-time PCR, which is a sensitive technique. However, some plants may contain components that can act as PCR reaction inhibitors, which can lead to false negative results or an underestimation of the bacterial concentration present in the analysed plant sample. Droplet digital PCR (ddPCR) is an innovative tool based on the partitioning of the PCR reagents and the DNA sample into thousands of droplets, allowing the quantification of the absolute number of target DNA molecules present in a reaction mixture, or an increase of the detection sensitivity. To improve Xf detection, the Harper et al. (2010) real-time PCR protocol, already used for surveys in several European countries, has been transferred to an optimised ddPCR protocol. This new assay was evaluated and compared with the initial real-time PCR on five plant matrices (*Lavandula angustifolia*, *Olea europaea*, *Polygala myrtifolia*, *Quercus ilex* and *Rosmarinus officinalis*) artificially inoculated and on naturally infected samples. This ddPCR assay enabled the detection of Xf in the five plant matrices artificially inoculated with a similar limit of detection, or a slight benefit for *Q. ilex*, compared with real-time PCR. Moreover, ddPCR improved diagnostic sensitivity as it enabled detection of Xf in samples of *P. myrtifolia* or *Q. ilex* that were categorised as negative or close to the limit of detection. This makes this ddPCR protocol the first one that can be used for the detection of Xf.

Poster

Implementation and validation of rapid diagnostic procedures for *Xylella fastidiosa*

Loconsole G*, Zicca S, Manco L, Altamura G, Abou Kubaa R, Potere O, EL Hatib O, Valentini F, Boscia D, Elicio V, Formica L, Savino VN, Saponari M

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Abstract: Surveys for *Xylella fastidiosa* are now mandatory in the EU Member States and genetically diverse strains, i.e. those capable of infecting different hosts, have been intercepted in the currently known EU outbreaks and/or containment areas. Given the long list of the EU susceptible host plants, currently comprising more than 50 plant species, there is an compelling need to develop robust and rapid diagnostic tests (ready-to-use and on-site tests) suitable for testing and screening different plant matrices and large numbers of samples. To this end, we have compared membrane-capture-based methods and ready-to-use kits, the latter based on isothermal nucleic acid amplification techniques. Indeed, a chloroform-free method based on the use of the Maxwell® RSC PureFood GMO and Authentication Kit (Promega) was evaluated on different plant matrices, as an alternative protocol for recovering total DNA suitable for identifying *X. fastidiosa* in real-time PCR (qPCR) reactions, so far the most widely used diagnostic method. In a first validation test, 90 olive field-trees were tested simultaneously using different procedures: FTA-ELISA, DTBIA, qPCR assay on membrane-captured DNA, loop-mediated isothermal amplification/LAMP on fresh sap or on stored tissue-imprinted membranes, and recombinase polymerase amplification/RPA. Performance criteria for each test were determined by comparing the results with those obtained in qPCR assays performed according to Harper et al. (2010). The results, while suggesting that different types of membranes can be used for capturing bacterial cells for a subsequent serological or molecular detection, showed that all these approaches produced lower accuracy values than LAMP, RPA and standard qPCR. The chloroform-free DNA purification kit herein tested made it possible to recover DNA templates of high quality with standardised yields, suitable for the detection of the bacterium using the qPCR protocols currently validated for *X. fastidiosa*.

Acknowledgement

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

Xylella fastidiosa: Vectors

Oral presentation

Mark–recapture experiments to estimate the dispersal capacity of *Philaenus spumarius*

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Abstract: The spread of the vectors is a key point in understanding the epidemiology of *Xylella fastidiosa* and in assessing vector control strategies. In Europe, the transmission of the bacterium is mainly due to spittlebugs. In particular, in the Apulia region (Italy) *Philaenus spumarius* has been proved to play the major role in transmitting *X. fastidiosa* subspecies pauca. Despite its importance, little information is available on the dispersal capacity of *P. spumarius*. To fill this knowledge gap mark-release-recapture experiments on *P. spumarius* adults were carried out in two agroecosystems: an olive grove and in a grass meadow, in the Apulia and the Piedmont regions (Italy), respectively. Dispersal capabilities of the vector were analysed in experiments performed from May to October in 2016 and 2017. Adults of *P. spumarius* of both sexes were captured in natural grassland habitats, marked with an aqueous solution of albumin and then released at a single point in the centre of the experimental area. The dispersal capacity was described estimating the probability density function describing the distribution of the end locations of insects relative to the source point (i.e. the dispersal kernel). Under the hypothesis of a random walk and applying a Gaussian kernel, diffusion rates in the two agroecosystems were estimated. Results showed a high variability in the estimated daily median distance from the release point, ranging from 19 to 51 metres. Considering that marked insects could disperse over an area wider than the experimental field, a correction for the truncated sampling bias has been included into the dispersal kernel, leading to a significant increase in the estimated daily median distances.

Oral presentation

Phenology and host-plant association of spittlebugs in Mediterranean olive groves

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Abstract: Phenology and ecology of *Philaenus spumarius* and other spittlebug species were investigated during regular field surveys in 2016–2018 in four olive orchards located in coastal and inland areas of the Apulia and Liguria regions of Italy, as part of an EFSA-funded project. The nymphal population in the herbaceous cover was estimated using quadrat samplings. Adults were collected by sweeping net on three different vegetational components: herbaceous cover, olive canopy and wild woody plants. Although the nymphs were polyphagous, they showed a strong host-preference for herbaceous plants of the Asteraceae and Fabaceae families in both the Liguria and Apulia regions of Italy: 72–88% of the total nymphs were indeed associated with these plant families. Nymphs of Aphrophora showed a similar host-preference, while those of Neophilaenus were strongly associated with Poaceae (85–100% of the nymphs were found on gramineous plants). Aphrophora alni and *N. campestris* showed a very low population density compared with *P. spumarius*. The average nymph population density of *P. spumarius* varied from 13 to 30 individuals/m^2 in Liguria according to the olive grove and the year, and from 5 to 19 individuals/m^2 in Apulia. Phenological data based on physiological time revealed that in Liguria the peak of abundance of the *P. spumarius* nymph population was between 150 and 210 degree day (DD) while in Apulia the same peak was between 100 and 270 DD. This difference among locations could be explained by a non-linear component in the temperature-dependent development rate function of *P. spumarius*. The phenological pattern in the two regions is more similar if referred to chronological time. In fact, nymphs developed in Liguria between early March and the end of May, and in Apulia between the end of February and mid-May. Field data are integrated with mesocosm and microcosm observations on the phenology and biology of *P. spumarius*.

Oral presentation

Use of vibrations to manipulate the behaviour of the meadow spittlebug *Philaenus spumarius*

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Abstract: Sexually mature adults of the meadow spittlebug, *Philaenus spumarius*, exchange vibrational signals through the host plants to communicate and achieve mating. Novel pest control strategies involve the manipulation of the sexual behaviour of the insect by means of species-specific mechanical stimuli transmitted to plants. Playback trials with mini-shakers were conducted to evaluate whether the transmission of pre-recorded *P. spumarius* vibrational signals to a plant of *Helianthus annuus* could affect the behaviour of the insect and to evaluate the potential use of vibrations for management practices against this pest. In all the trials, vibrational signals emitted by the specimens were recorded with laser vibrometer. At the beginning of the season (June–July), the natural female rejection signal (FRjS) was tested on males (n = 30) to assess a possible repelling effect from the plant, thus reducing their feeding time and the associated inoculation of *Xylella fastidiosa* to olive trees. Similarly, young males (n = 20) and young females (n = 20) were tested with playback of the male calling signal to ascertain the behavioural role of this signal spontaneously emitted by males since their emergence. Playback of the mating duet was played to evaluate a possible attraction effect on males (n = 30) to the source of the signal, triggered by their satellite behaviour. In the late season (August and September), pairs consisting of a female and a male were released on different leaves of an *H. annuus* plant and stimulated either with the playback of the male rivalry signal (n = 30) or the FRjS (n = 30) to disrupt the pair-formation process. Again, the FRjS (n = 30) was tested for the potential suppression of the spontaneous female calling activity. Results of all the playback trials are presented as well as a description of the mating behaviour. Insights on the potential future development of sustainable control strategies against this pest are given

Oral presentation

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

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Abstract: The establishment and relentless spread of the bacterium *Xylella fastidiosa* in some areas in Europe call for effective containment measures based on sustainable control strategies. However, the development of such strategies requires a thorough characterisation of the reciprocal interactions among the three key factors of the pathosystem, i.e. the bacterium, the vector, and the host plant. One of the major differences between European and American or Taiwanese epidemics refers to the vector species driving bacterium spread. Indeed, while sharpshooters (Hemiptera: Cicadellidae) are the key vectors in all the *X. fastidiosa* outbreaks other than European ones, spittlebugs such as *Philaenus spumarius* seem to play the main role in bacterial spread in Europe. Currently, knowledge about *X. Fastidiosa*–spittlebug interactions and the characterisation of the mode of transmission considerably lags behind the background on sharpshooters. Here we began to fill this knowledge gap by carrying out EPG (Electrical Penetration Graph)-assisted transmission tests of *X. fastidiosa* by *P. spumarius* (acquisition from infected olive plants and inoculation of healthy olives and oleanders). Furthermore, we conducted comparative observations on the probing and feeding behaviour of infective versus non-infective spittlebugs on healthy olive plants. The spittlebug acquisition rate of *X. fastidiosa* from olive appeared to be extremely low; bacterial cells binding to the foregut occurred in a time as short as 15 minutes spent by the insect in xylem ingestion or activities interspersed with xylem ingestion (interruption during xylem ingestion and resting). Either in olive or oleander, *P. spumarius* inoculation of bacterial cells into the xylem was associated with an early (2.5 to 7 minutes after the onset of the first probe) and occasional behaviour, visualised by a specific DC-EPG waveform (Xe), presumably related to egestion of fluids regulated by pre-cibarial valve fluttering following a lack of phagostimulation. Behaviours stereotypically repeated by the insect and commonly performed during most of the probes did not lead to bacterial inoculation of the host plant. Infective spittlebugs compared with non-infective spittlebugs exhibited: i) significantly longer non-probing and shorter xylem ingestion; ii) longer duration of single non-probing events; iii) fewer sustained ingestions (ingestion longer than 10 min) and interruptions of xylem activity; iv) longer time required to perform the first absolute probe. These observations suggest difficulties in feeding for infective *P. spumarius* probably caused by the presence of *X. fastidiosa* in the foregut. Overall, our findings open new perspectives for research on the *X. fastidiosa*–spittlebug relationship and for sustainable control strategies based on the disruption of the bacterium–vector interaction.

Oral presentation

Detection, identification and surveillance of *Xylella fastidiosa* on vectors in France

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**ANSES, Plant Health Laboratory, Angers (FR)*

Abstract: *Xylella fastidiosa* was identified in natural conditions in France in 2015, first on Corsica, then in the Provence-Alpes-Côte d'Azur administrative region (French Riviera). Forty-nine host plants have now been identified, infected by *X. fastidiosa* subsp *multiplex*.

In France, the detection protocol on vectors is based on the real-time PCR Harper et al. (2010) after a high-throughput DNA extraction based on the QuickPick™ Plant DNA kit used with a KingFisher™ robot. This method was evaluated in 2016–2019 mainly on *Philaenus spumarius*, the most effective vector based on POnTE studies from CNR. This protocol showed excellent performance criteria on individuals and groups of insects, on spiked macerates of insects from healthy areas and on naturally infected insects from outbreaks areas.

Strain identification was performed using an MLST scheme (www.pubmlst.org) with the PCR Yuan et al. (2010) (modified EPPO, 2019) for amplifying sequences of seven housekeeping genes. A protocol with the addition of BSA into the PCR reaction mixture has been validated, showing improved performance in terms of sensitivity and success for strain typing. The centralisation of surveillance data at Anses enabled the production of zonal maps of the distribution of strains according to host species. They have been compared with compilations of results of detection and strain identification on insects *Philaenus spumarius* from Corsica and the French Riviera regions. A good correlation between both maps has been observed in the same area. This work highlights the complementarity of the approaches on plants and insects in the context of the epidemiological survey of the disease in a situation of high risk for Europe.

Oral presentation

Transmission characteristics of *Xylella fastidiosa* subsp. pauca (ST53) by *Philaenus spumarius* and *Cicadella viridis*

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

Abstract: For insect-borne plant pathogens, transmission biology is of major importance in outlining the disease epidemiology. The characteristics of acquisition, persistence and transmission of *X. fastidiosa* ST53 by the spittlebug *Philaenus spumarius*, the main vector in Apulia, are not yet described; similarly, transmission competence of the potential vector *Cicadella viridis*, the most common sharpshooter in Europe, is unknown. In this perspective, two sets of experiments were performed in 2017 and 2018 to study: i) the kinetics of bacterial multiplication and persistence in *P. spumarius* and *C. viridis*; ii) the influence of temperature, season and age of *P. spumarius* adults on simulated epidemic progression on olive plants under indoor and outdoor conditions.

For the kinetics experiments, following the acquisition, insects were serially transferred in groups of five to olive or periwinkle test plants. In simulated epidemic progression experiments, after the acquisition, groups of insects were isolated in cages with 16 olive seedlings for different inoculation periods. Acquisition and transmission rates were assessed by testing individual insects after inoculation and by testing recipient plants 6 and 10 months post-inoculation. Furthermore, acquisition and transmission of *X. fastidiosa* ST53 by *C. viridis* were tested through an *in vitro* acquisition system. Overall, about 900 insects and 170 plants were tested in kinetics experiments, while about 800 spittlebugs and 1,500 plants were studied in simulated epidemic progression experiments.

Preliminary results for *P. spumarius* indicate: a) a higher acquisition efficiency in September than July; b) a lower acquisition efficiency from periwinkle compared with olive as source plants, but higher transmission efficiency to periwinkle compared with olive as recipient plants. *Cicadella viridis* was able to acquire and transmit *X. fastidiosa* following acquisition on artificial diet or periwinkle, although with low efficiency.

Oral presentation

Flight behaviour of *Philaenus spumarius*, the main vector of *Xylella fastidiosa*

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Abstract: *Xyllela fastidiosa* jeopardises key crops in Europe. *Philaenus spumarius* was identified as the predominant vector involved in the spread of *X. fastidiosa* in southern Italy. This meadow spittlebug is also distributed in other regions, including Spain. Understanding vector dispersal ability is essential to predict the spread of *X. fastidiosa*. This insect species was reported to travel as much as 100 m within 24 hours in the field. However, both *P. spumarius* and *Neophilaenus lineatus*, another potential vector, can vertically displace up to the planetary boundary layer so they may passively travel long distances by laminar air currents. Our goal was to study the movement of *P. spumarius* under laboratory conditions using a modified commercial flight mill. Field studies on vector movement using vertical sticky traps; directional malaise traps and capture-mark-recapture techniques are underway. Different biotic and abiotic factors affecting the flight behaviour of *P. spumarius* are being studied: gender, adult age, temperature, light, barometric pressure, seasonality, geographic origin and rearing conditions among other factors. The individuals were tested using flight mills to estimate the number of flights, flight duration and number of turns (distance). The number and duration of turns were recorded using two different procedures: 1. Ethovision XT (Noldus), placing a video camera above the flight mill; 2. Mill_recorder, a computer-based device programmed to register the number and duration of each turn. Our data available to date show that *P. spumarius* is able to fly a distance of at least 1.99 km in 1 h 40 min in a single flight, which is much higher than was previously thought. Furthermore, our preliminary results show that there are differences in the flight potential between males and females and between young and old adults. This knowledge on the flight potential of *P. spumarius* will be critical to improve management actions against the vector and the spread of *X. fastidiosa* in Europe.

Oral presentation

Host plant affiliation of xylem-feeders in central Europe

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Abstract: Provoked by the first notification of *Xylella fastidiosa* in Europe, several countries aimed to acquire data on the presence of already confirmed as well as potential vector species of the bacterium present in Europe.

In Germany, vector surveys were carried out predominantly on plant species which are economically important in central Europe and known to be highly endangered by *X. fastidiosa* in the event of further bacterial spread, like grapevine, almond and cherry. Results are shown, which have been obtained from a three-year survey in the course of the project Xf-actors. The population dynamics of xylem-feeding species (spittlebugs, froghoppers and sharpshooters) were assessed in orchards (cherry and almond) and vineyards. Furthermore, the xylem-feeding activity of the most prevalent species on these plants was observed by EPG (Electrical Penetration Graph) to evaluate their potential vector-related risk as host for *X. fastidiosa* acquisition. The results will support an estimation of the transmission risk to specific crops under field conditions with a special focus on species able to transmit the bacterium, like *Philaenus spumarius* and *Neophilaenus campestris*.

Poster

Vector parameters relevant to model the management of *Xylella fastidiosa pauca* ST53 invasion

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Abstract: We present a model, based on both Life Table and Statistical Mechanics techniques, aimed at identifying a valid Integrated Pest Management strategy to mitigate or stop the *Xylella fastidiosa pauca* (Xfp) ST53 OQDS invasion. The model represents closed and open systems as simple square lattices that mirror actual olive orchard structure, with different boundary conditions. Main orchard parameters involved in the model are: trees per ha, length, number of branches and twigs, olive susceptibility to vector infestation and Xfp ST53 propagation rate in xylem. Vector parameters belong to the bionomics of *Philaenus spumarius* (Hemiptera Aphrophoridae) the main Xfp ST53 vector and are: population size, prolificacy, post-embryonic death rate, time to eclosion, feeding time, active dispersion, vector infection probability. Control parameters focus on juvenile vector control, adult vector control, control action timing and frequency, treatment efficacy, vector survivorship. Vector population control is performed through specific egg and juvenile population management actions, diminishing the overall population size. Transmission management is performed by means of chemical actions (i.e. spray or injection of synthetic xylem-moving insecticides, selected by experimental mortality data) that kill the adults during the acquisition on the tree and minimise the following infection spread. The control of adults is challenging because *P. spumarius* acquires and performs infections and further transmissions almost without latency, depending on the status of the plant they encounter. The impact of orchard structure, number and frequency of different means of discouraging or impeding the transmission are also discussed. This study shows that different tailoring, timing and tuning of available control actions lead to different invasion control efficacies. Nevertheless, harmonising within an IPM strategy the control of both vector population and transmission can mitigate the infection, ending the pathogen invasion, eventually.

Poster

Relationship between vectors of *Xylella fastidiosa* and the almond leaf scorch disease in the demarcated area in the province of Alicante (Spain)

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Abstract: *Xylella fastidiosa* was identified for the first time in mainland Spain in 2017, in the northeast of Alicante province, on almond trees affected by leaf scorch. Since then, the disease evolution and the potential vectors of the bacteria have been monitored periodically in the demarcated area. This work shows the updated results on the surveillance and analysis campaign carried out from 2017, based on the sampling of almond trees and nymphs and adults of potential vectors, captured both on the vegetation cover and on the trees from several orchards. The vector species present in the area have been identified, and both the insects and their host plants have been analysed for *X. fastidiosa* detection.

Until now, *X. Fastidiosa* has been detected in two insect species, which some authors have previously shown to transmit the pathogen: *Philaenus spumarius* and *Neophilaenus campestris*. The relative presence of the two vectors in the samplings was similar: 42% for *P. spumarius* and 54% of *N. campestris*. However, the bacterial infection percentage among the analysed individuals was quite different: 10.49% in *P. spumarius* and 0.75% in *N. campestris*.

With global results, the relationship between these potential vectors and the distribution of the infection in the different zones of the demarcated area, are being analysed.

Poster

Distribution and identification of potential vectors of *Xylella fastidiosa* in almonds, vineyards and citrus in mainland Spain

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Abstract: The introduction of the vector-borne bacterium *Xylella fastidiosa* on the European continent is causing important economic and social disturbances, especially among the olive groves of southern Italy. The recent detection of the pathogen in several key crops in mainland Spain such as almond, apricot and plum in Alicante and in olive in Madrid has increased the need to assess the degree of threat to which Spanish agriculture is exposed. Therefore, a deep knowledge about the distribution and population trends of potential vectors (Hemiptera: Cicadomorpha) is required to assess the risk of pathogen spread. In addition to the recently published results in olive groves, almond groves (Madrid and Alicante), vineyards (La Rioja and Madrid) and citrus groves (Murcia) were sampled from 2017 to 2019 in order to identify the population trends of the potential vectors of *X. fastidiosa*. Nymphs of spittlebugs were sampled every 10 days starting from early March until spittles were no longer observed. The number of spittles per plant, number of nymphs per spittle, and spittle position on the ground vegetation was counted. Adults of Cicadomorpha were sampled both in the ground vegetation and the tree canopy by using the sweep net method from early April to November. The spittlebugs *Philaenus spumarius* and *Neophilaenus campestris* (Hemiptera: Aphrophoridae) were the main vector species associated with the three crops. Furthermore, we found the potential vector *Cicadella viridis* (Hemiptera: Cicadellidae) on the ground vegetation associated with the vineyards located in La Rioja (northern Spain). Preliminary results indicated that the highest vector density was observed in almond groves of Alicante. We found a high density of nymphs of *P. spumarius* and *N. campestris* from early March to late April reaching densities higher than 100 nymphs/m^2 in ground vegetation below the almond trees. However, the rest of the areas sampled presented a much lower density of vectors ranging from peaks of 1.3 nymphs/m^2 in almonds to 4.8 nymphs/m^2 in vineyards.

Poster

Improvement of a real-time lamp protocol for the detection of *Xylella fastidiosa* in *Philaenus spumarius* and *Neophilaenus campestris*

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Abstract: The epidemic spread of *Xylella fastidiosa* (Xf) in southern Italy, with very important economic repercussions for the olive tree industry, makes it advisable to use methodologies for early monitoring of potential tree infection before symptom development in host plants, such as the use of spy insects. This approach is based on the use of molecular tests to detect the presence of Xf, among which the real-time LAMP. In this work, a commercial kit (Enbiotech, Italy) based on this technique, was assayed for detection of Xf in *Philaenus spumarius* and *Neophilaenus campestris* specimens in different demarcated areas in Europe. Spiked samples were tested using the entire insect and bulk insect heads artificially inoculated with serial dilutions (from 106 to 100 CFU) of a strain of *X. fastidiosa* subsp. *pauca* ST53, isolated from an olive tree in Apulia. In order to exclude the loss of sensitivity due to the presence of inhibitors in the reaction, spiked samples were also tested using the insect heads macerated in the extraction buffer provided by the kit. The lowest bacterial dilution was always detected. Approximately 525 individuals of *P. spumarius* were sampled in late summer in an infected olive grove in Lecce (Italy) and tested by this assay. The total incidence of infection ranged from 13% to 16%, using single entire insects or single heads, respectively. This incidence was confirmed with bulk heads to assess the diagnostic sensitivity of the real-time LAMP test; with a progressive increase in bacterial detection observed by analysing a higher number of heads. Finally, heads of approximately 280 *P. spumarius* and *N. campestris*, collected in an infected almond grove in Alicante (Spain), are being analysed to determine the infection prevalence using the commercial kit and the real-time-PCR of Harper et al. (2010, erratum 2013), to compare the sensitivity of both techniques.

Poster

What are the potential vectors of Xylella in France? Overview of the results of a trapping network 2017–2018

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Abstract: From 2017 to 2018, Anses-LSV has implemented a national network for sampling potential vector insects of *Xylella fastidiosa*. Insects were collected in cultures and their nearby environment by different partners. About 385 sites were monitored with 1,563 samplings (all origins and years combined) in 10 of the 13 regions in metropolitan France. The top three participating regions are Occitanie, Nouvelle Aquitaine and Provence-Alpes-Côte d'Azur.

Samples were taken in agricultural crops and in the immediate environment/hedges. Several partners also carried out occasional samplings in non-agricultural or other areas.

When the location of the sampling is known, the main crops visited are grapevine (38%), and orchards (20%). Other samples include other crop types (including olive groves) but also uncultivated sites (e.g. scrubland, forest edges, lawns). The main technique used is the sweeping net in most situations.

A total of 31,207 insects (larvae and adults) were collected, including a majority of insects belonging to potential vector families. They were identified by morphological means. After exclusion of larvae (that can often not be determined morphologically) and cicadellid subfamilies not xylem-feeders (12 of 15 subfamilies in Europe), more than 9,000 potential vectors were studied.

The most common potential vectors in France are *Philaenus spumarius*, *Cicadella viridis*, *Neophilaenus campestris* and *Cercopis vulnerata*. We confirm here that *P. spumarius* is the most common species in France amongst potential vector species. We tested four methods to collect insects in the fields. No method is specific to Xf vectors. The method which combines the most abundant harvest and specificity, is the sweeping net. The Barber trap is only suitable for leafhoppers living at the soil surface and yellow pan traps and sticky traps are not sufficiently specific. Based on the network, we also provide basic phenology for the most common species.

Poster

Seasonal occurrence of *Philaenus spumarius* and *Neophilaenus campestris* in olive orchards of Greece

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Abstract: The introduction of the xylem-feeding bacterium *Xylella fastidiosa* in Europe has elicited investigations about the ecology of insect vectors that are potentially associated with the spread of the disease. In 2018 and 2019 samplings were carried out in olive orchards of central (Corinthia) western (Achaea) and southern (Chania, Crete) Greece to investigate: i) the seasonal occurrence of potential vectors of *X. fastidiosa* in Greece, ii) to identify host plants associated with the nymph's development. Samplings for nymphs are performed by collecting plants with spittle within a quadrate frame. Adults are collected using a sweep net and samples are taken both from the olive tree's canopy and ground vegetation.

In the areas of central and western Greece, *Philaenus spumarius* and *Neophilaenus campestris* nymphs were found on plants with spittle from mid-March until the end of April in 2018. In Chania Crete, only nymphs of *P. spumarius* were observed and they were present from late March until late April. Adults of *P. spumarius* and *N. campestris* were present in the areas of central and western Greece during April and May. However, during the summer months (June–September) they were absent from those olive orchards. They reappeared in early October and were captured until the end of December in those areas. In the sampling orchard of central Greece a few spittlebug adults were also captured during the winter months (January and February 2019). In the area of southern Greece, adults of *P. spumarius* were observed in April and from late October until mid-December. Regarding host plants, *P. spumarius* nymphs were observed mostly on plants of the Asteraceae and Fabaceae families while *N. campestris* nymphs mostly on plants of Poaceae family. Samplings for 2019 are ongoing.

Poster

Electrophysiological responses of insect vectors of *Xylella fastidiosa* to plant volatiles

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Abstract: The spittlebugs *Philaenus spumarius* and *Neophilaenus campestris* (Hemiptera: Aphrophoridae) are xylem-feeder insects that have been identified as vectors of *Xylella fastidiosa*. Plants emit a variety of volatile organic compounds (VOCs) that play multiple roles in plant–insect interactions; these semiochemicals are important cues for insects to locate an appropriate host plant, or mating and oviposition sites. Since spittlebugs have never been considered agricultural pests in Europe before the introduction of *X. fastidiosa*, their biology and ecology have been scarcely studied. So far, no research has been conducted to examine the important ecological chemistry behind these host plant–insect systems. In olive orchards in Greece, *P. spumarius* nymphs have been observed mostly on plants of the Asteraceae and Fabaceae families while *N. campestris* nymphs mostly on plants of the Poaceae family. In the summer months, adults of both species move from grass cover to woody plants and move back to grass cover in autumn. Using the dynamic headspace technique, we collected VOCs from *Olea europaea*, the major host plant for *X. fastidiosa*, and from various 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* and *Sonchus oleraceus*. Additionally, we collected VOCs from *Pinus halepensis* that is common in the edges of olive plantations and from *Polygala myrtifolia* which is a highly susceptible plant to *X. fastidiosa*. Electrophysiological bioassays of both *P. spumarius* and *N. campestris* adults to the collected VOCs are scheduled using gas-chromatography coupled with electro-antennographical detection (GC-EAD). Additionally, specific electrophysiological responses (EAG) of the insect's antenna to single chemicals identified in the volatile blends of the aforementioned plants are ongoing. Subsequently, the compounds that elicit a reaction in GC-EAD and EAG assays will be tested in behavioural experiments. The identification of semiochemicals that manipulate the spittlebug behaviour could contribute to the development of efficient monitoring tools for *X. fastidiosa* vectors.

Poster

Assessment of the genetic diversity in populations of *Philaenus spumarius* collected from different areas

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Abstract: The spittlebug *Philaenus spumarius* is a widespread species in Europe, recently attracting attention because of its role in spreading the plant pathogenic bacterium *Xylella fastidiosa* (Xf), a new emerging pathogen threatening the landscape and several crops in southern European countries. Being the main species associated with the epidemic spread of this bacterium currently decimating olive trees in the Apulia region (southern Italy), molecular investigations have been carried out to characterise the populations occurring in the so-called *Xylella*-demarcated areas of Apulia versus those present in other regions currently free from *X. fastidiosa*. Analysis also included specimens received from Montenegro and Tunisia.

Three DNA markers were used, the mitochondrial genes COI and cytochrome b and the nuclear gene EF1-α, to amplify and sequence DNA fragments from a total of 60 specimens. Mitochondrial sequence analysis showed that all Italian specimens belong to the south-west clade (which includes the Mediterranean basin and western Europe) and, in particular, the majority of the Apulian specimens and those collected in Montenegro belong to the eastern Mediterranean subgroup, whereas the specimens collected in other regions of Italy belong to the western Mediterranean subgroup. Conversely, the Tunisian specimens fell in the eastern group (Anatolia/Caucasus). Similar genetic relationships were retrieved from the analysis of the EF1-α gene.

The data herein obtained, while confirming that the population of *P. spumarius* responsible for the epidemic spread of Xf in Apulia belong to a single phylogenetic group, integrate the large dataset of biological and ecological information collected from the studies intensified on this species in the last few years.

Acknowledgement

The authors would like to thank the project 'Capacity Building and Raising Awareness in Europe and in Third Countries to Cope with *Xylella fastidiosa* - CURE-XF' the European Union's H2020-MSCA-RISE-2016 under grant agreement N. 734353 for its partial support of this work.

Poster

Predominance and natural infectivity of potential vectors of *Xylella fastidiosa* in olives in south-eastern Brazil

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Abstract: As xylem-feeders, several species of sharpshooter (Hemiptera: Cicadellidae: Cicadellinae) and spittlebug (Hemiptera: Cercopoidea) are potential vectors of the xylem-limited bacterium, *Xylella fastidiosa*, which is associated with olive quick decline syndrome symptoms in south-eastern Brazil. Here we investigated the epidemiological importance of these species based on faunal indices and natural infectivity with *X. fastidiosa* in olive orchards of the Mantiqueira Moutain Range (MMR) region, in São Paulo (SP) and Minas Gerais (MG) states. The insects were sampled with yellow sticky cards hung at two heights (0.8 and 1.6 m) on the outer branches of nine olive trees per orchard; the cards were replaced fortnightly from June 2015 to August 2017. Based on faunal analysis, those species classified as dominant, very abundant and very frequent were considered predominant. Samples (n=15) of three individuals of each predominant species were evaluated for the presence of *X. fastidiosa* by qPCR and conventional PCR. Out of 48 species (5,055 individuals) of sharpshooters and spittlebugs trapped in an orchard in São Bento do Sapucaí (SP), six are predominant (*Clastoptera* sp.1, *Amblyscartidia pardaliota*, *Macugonalia cavifrons*, *Paratubana luteomaculata*, *Scopogonalia paula* and *Subrasaca bimaculata*), whereas in an orchard in Maria da Fé (MG), 7 of 45 species trapped (2,026 individuals) are predominant: *Clastoptera* sp.1, *Bucephalogonia xanthophis*, *Erythrogonia dorsalis*, *Erythrogonia phoenicia*, *M. cavifrons*, *S. paula* and *Oncometopia facialis*. *X. fastidiosa* was detected in samples of *Clastoptera* sp.1 (53%), *M. cavifrons* (20%), *P. luteomaculata* (50%), *S. paula* (20%) and *S. bimaculata* (33%) collected in São Bento do Sapucaí, but in none of the four predominant species collected in Maria da Fé. Combined results of predominance and natural infectivity show that the spittlebug *Clastoptera* sp.1 and the sharpshooters *M. cavifrons*, *P. luteomaculata*, *S. paula* and *S. bimaculata* may be epidemiologically relevant for disease spread in the MMR region, if confirmed as vectors of *X. fastidiosa* in olives.

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

Poster

Abundance of spittlebug nymphs (Hemiptera: Aphrophoridae) in Trás-os-Montes region, Portugal

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Abstract: Spittlebugs (Hemiptera: Aphrophoridae) are considered the main European vector of *Xylella fastidiosa*, a gram-negative bacterium responsible for serious diseases in important agronomic crops. Nymphs of spittlebugs develop in vegetation cover where they produce a spittle mass that provides protection from natural enemies and solar radiation. This spittle mass is visible to the unaided eye which facilitates its monitoring, the understanding of nymph's dynamics and the implementation of control strategies against *X. fastidiosa*. In this context, the goals of this work were monitoring the abundance of spittlebug nymphs and identifying the host plants in the natural ground cover in the Trás-os-Montes region, Portugal. For that, the presence, number of spittles per plant, number of nymphs per spittle, and spittle position low, medium or high were recorded over a sample unit of 100 × 25 cm in one olive grove (spring 2017 and 2018), in one almond orchard and one vine (spring 2018) from Trás-os-Montes. Thirty sample units randomly distributed over a transect covering 1 ha were selected per sampling date and sampling site. The peak of spittlebug nymphs occurred in the middle of April and first weeks of May in 2017 and 2018, respectively, in all sampling sites. Nine spittle masses and a mean of 1.93 ± 0.35 plants with spittle per m^2 were recorded respectively in 2017 and 2018. In 2017, *Crepis vesicaria* L. and *Bromus diandrus* Roth were the most attacked species. In 2018, the presence of nymphs of the genus *Neophilaenus* was more abundant in the vine, presenting a mean of 0.60 ± 0.19 nymphs per m^2, while *Philaenus* nymphs showed a higher abundance in the olive grove, presenting a mean of 0.17 ± 0.08 nymphs per m^2. Nymphs of the genus *Neophilaenus* were identified with high frequency in the species *Avena barbata* subsp. *lusitanica* (Tab. Morais) Romero Zarco and *Cynodon dactylon* (L.) Pers and the *Philaenus* nymphs were identified more frequently in the species *Erodium cicutarium* (L.) L´Her. and in the genus *Trifollium* spp. It was verified that the number of nymphs registered in the Trás-os-Montes region was much lower than the numbers reported in other European regions, necessitating more years of study to understand the dynamics of spittlebug nymphs.

Poster

Surveys for vectors and candidate vectors of *Xylella fastidiosa* in olive orchards in Apulia

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Abstract: For three consecutive years, surveys were conducted in 24 olive orchards located in the demarcated *Xylella*-infected area of Apulia (southern Italy), with the aim of identifying xylem-feeder species serving as vector of *Xylella fastidiosa* in olives. Surveys were carried out periodically from spring to late autumn and insects were collected by sweeping net using constant sampling units from olive canopies, ground vegetation and border plants when present. All insects were subjected to qPCR for the detection of *X. fastidiosa* (individually or in groups of 5), as well as to the morphological identification. Besides the two spittlebug vectors (*Philaenus spumarius* and *Neophilaenus campestris*), more than 3,000 specimens belonging to 37 different species (27 Cicadomorpha and 10 Fulgoromorpha) of 34 genera and 10 families were collected. The detection of the bacterium in spittlebugs, xylem-feeders and on some of the most abundant phloem-limited species, showed that: (i) for the two known vectors occurring in the area, a higher incidence of positive specimens was detected for *P. spumarius* (up to 50%) with the occurrence of *Xylella*-positive insects for a prolonged period, compared with *N. campestris* (10% of positive specimens, detected to a limited extent in late spring), and (ii) for both species the incidence of positive specimens was consistently higher when they were collected from olive canopies compared with ground vegetation or border plants; (iii) amongst the xylem-feeders, none of the specimens collected and tested for *Cicada orni*, *Cercopis sanguinolenta* and *Lepyronia coleoptrata* were found positive; (iv) conversely, few positive specimens were recovered for the following leafhoppers and planthoppers: *Thamnotettix zelleri* (8.33% in olives and 3.7% in weeds); *Latilica tunetana* (1.77% in olives, 2.2% in weeds) and for *Euscellis lineolatus* (none in olives, 0.06% in weeds). Regarding the possible role as vector of *X. fastidiosa* for these planthopper/leafhopper species, transmission tests have already excluded the capability of the *L. tunetana* to retain and transmit the bacterium, whereas experimental tests are ongoing for *T. zelleri*.

Acknowledgement

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Xylella fastidiosa: Ecology, epidemiology and modelling

Oral presentation

Temperature determines growth and biofilm formation of *Xylella fastidiosa* strains *in vitro*

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Abstract: *Xylella fastidiosa* (Xf) is classified as a mesophilic bacterium particularly well adapted to areas with mild winters. The effects of temperature on Xf development have been addressed only for Xf subsp. *fastidiosa* strains. In this work, we have explored the effects of temperature on growth and biofilm formation *in vitro* for Xf strains representative of Xf subspecies prevalent in the European outbreaks. Growth, biofilm formation and cell viability of Xf strains from subsp. *fastidiosa* (7 strains), *multiplex* (18 strains), *pauca* (3 strains) and *sandyi* (2 strains) of different geographic and host origin have been determined in microplates with PD3 medium incubated at a constant temperature range of 6–32 °C at 4 °C intervals. Growth was determined daily by optical density at 600 nm, and biofilm was assessed at the end of the experiments by a crystal violet assay. Both parameters were modelled using a beta function to estimate the cardinal and optimal temperature values for each strain. Our results indicate that both growth and biofilm formation is determined by temperature and Xf strain. Overall, minimum vales for both parameters were observed at the extreme temperatures of 6 and 32 °C. However, the range with optimal values largely depends on the Xf strain. Although no distinct range was associated with subspecies, the widest temperature range for optimal growth was estimated for Xf subsp. *multiplex* strains at 20–32 °C. Optimal growth was estimated at 24–32 °C for Xf subsp. *fastidiosa* strains. No clear pattern was found for Xf subsp. *pauca*, with optimal values between 16 and 24 °C or 20 and 28 °C, depending on the strains. Similarly, for Xf subsp. *Sandyi*, optimal growth occurred at 16–28 °C or 20–28 °C. With a few exceptions, similar response to temperature was observed for biofilm formation.These results would allow the development of region-specific epidemiological models to predict the risk associated with the establishment and spread of Xf strains in Europe.

Acknowledgement

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Oral presentation

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

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Abstract: 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, which have been 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 try to estimate long-distance dispersal, we use Approximate Bayesian Computation to calibrate the epidemiological model to observed detections in surveillance monitoring data from 2013 to 2018. I will present results from the model calibration, comparing long-distance dispersal estimates from models specified for different long-range dispersal mechanisms. This will inform discussion on the roles of mechanisms such as vehicle transport and wind dispersal in spreading *Xylella* at regional scales.

Poster

Modelling the spread and control of *Xylella* in novel outbreak locations

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Abstract: Predicting the spread of potential *Xylella fastidiosa* outbreaks in novel locations is of general interest in pest risk assessment throughout Europe. However, translating our knowledge of the disease to novel locations can be problematic due to the potential differences in environment and epidemiology. In turn, it is unclear how these differences affect the effectiveness of prescribed containment and eradication strategies. To address this question, we present a model which simulates spread by coupling a generic epidemiological model with a dispersal–kernel model. Available data on the epidemiology and spread of *X. fastidiosa* in Apulia, Italy, are used to parameterise the model but the influence of changing the epidemiological and landscape parameters is also assessed. The model illustrates the effectiveness of the current measures in limiting further spread, and in some cases, reversing the expansion of infected areas and even eradicating *X. fastidiosa* outbreaks. Reducing buffer zone width in both containment and eradication scenarios increased the area infected. The importance of early detection of new outbreaks is key to successful control, demonstrating the importance of increased surveillance and detection capabilities. In addition, we present our preliminary results on the potential spread and control of *X. fastidiosa* outbreaks in the UK.

Poster

Risk-based surveillance strategies for early detection of *Xylella fastidiosa* in continental France

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Abstract: *Xylella fastidiosa* has only recently been detected in metropolitan France, specifically in the southern region of Provence-Alps-Côte d'Azur. The threat posed by the potential spread of the bacterium to other parts of the country pushed national plant protection agencies to implement a monitoring campaign across the entire territory, with a special focus on those areas where the disease had already been detected. However, the sampling and monitoring effort that is still necessary to assess the actual dissemination of the bacteria is high, with many parts of the country still poorly sampled. In this work, we propose different surveillance strategies that aim at choosing the most effective areas where sampling and monitoring should be performed in order to detect the presence or arrival of the pathogen. These strategies are based on detailed risk maps of potential infection that have already been produced by the authors in another contribution. Our methodology involves the use of simulated epidemics on a spatial network representing the entire French territory at a fine spatial resolution and estimates the appropriateness of the different strategies according to different measures of efficiency in early detection. Finally, we will test different scenarios according to increasing sampling efforts that the surveillance agency may be willing to make and we will measure the marginal gain in detection for every added unit of sampling.

Poster

Bayesian analysis of climatic and spatial factors on *Xylella fastidiosa* distribution in the demarcated area in Alicante (Spain)

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Abstract: Almond leaf scorch, caused by *Xylella fastidiosa* subsp. *multiplex* (Xfm), was detected in 2017 in Alicante (Spain). Since then, intensive surveillance has been carried out in the demarcated area. In this study, the effect of climatic and spatial factors in the geographic distribution of the pathogen was analysed. In addition, it was studied whether the risk levels based on the minimum winter temperatures, defined by A. H. Purcell for *X. fastidiosa* subsp. *fastidiosa* in grapevine in North America, can be extrapolated to Xfm in almond in Alicante. Average monthly climate data for 1970–2000 was obtained from the WorldClim version 2 database. Presence/absence data of Xfm in the official surveys in the demarcated area in Alicante in 2017 were analysed using a Bayesian approach, through the Integrated Nested Laplace Approximation. An advantage of Bayesian hierarchical spatial models is that they allow the spatial autocorrelation that exists between the geographical locations of the observations to be taken into account while providing a more realistic and accurate estimation of uncertainty. This spatial effect was included through a conditional autoregressive structure. Climatic factors were not relevant in the model, likely due to the reduced geographic extent of the study area and the resulting limited variability of climatic covariates. Nevertheless, Xfm was detected within all minimum winter temperature thresholds defined by A. H. Purcell (from < 1.1 °C to > 4.5 °C), illustrating the known climatic adaptability of Xfm. A strong effect of the spatial component was obtained in the models. That is, the disease spread was largely defined by the spatial relationship among geographic locations.

Poster

Integrating spread modelling and remote sensing imagery to optimise early detection and spatial distribution estimation of *Xylella fastidiosa*

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Abstract: The long list of potential host species for *Xylella fastidiosa* (Xf) and its more recent detections in France and Spain demonstrate the potential for further pathogen spread throughout Europe, which has highlighted the importance of surveillance for protection of host plants against this pathogen. Xf is difficult to manage once it has become established in a territory, so effective surveillance efforts must be focused on the early detection for its erradication in new areas. However, the detection of early infections or even the spread of an establishing epidemic are limited by the cryptic nature of Xf. Thus, the effective deployment of methods to identify early infection of Xf is currently an important and innovative area of research. Epidemiological models have been used to predict pathogen spread and test surveillance strategies, but they rely on parameters estimated from sparse and imperfect data collected via ground surveys with limited budgets. In this sense, remote sensing (RS) has shown potential as a new form of large-scale surveillance, being able to identify pre-visual stages of disease development. However, RS information does not account for the connectivity of host landscape and the impact that the connectivity of susceptible host populations has on disease dynamics. This study demonstrates how combining epidemiological models, which capture the impact of connectivity and spatial disease dynamics, with RS, obtain better predictions of spatial disease distribution than both methods separately. The estimation method is based on an iterative stochastic optimisation algorithm to estimate the spatial distribution of Xf infection from a sample by explicitly simulating the individual distance-dependent spread processes between the pathogen and its host population. The present study adds some innovative aspects to this approach: the contribution of host connectivity on the pathogen spread throughout the landscape by circuit and graph theories and RS-based infection estimations using data provided by XF-ACTORS.

Poster

Modelling xylem temperature in olive and almond trees to estimate *Xylella fastidiosa* infection in woody hosts

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Abstract: *Xylella fastidiosa* (Xf) can infect a wide range of woody crops that are grown extensively worldwide. The temperature patterns inside the woody plant parts which are the habitat of Xf cells may nevertheless be different to those of the standard recorded air temperature, due to the wood thermal inertia, wind speed or incident radiation energy and its shading by the canopy. To investigate the relationship between the temperatures that can be reached in different parts of the xylematic system and those of the surrounding air, an experiment was conducted in Córdoba, southern Spain, from 2017 to 2019. In a high-density plot, a set of four mature olive trees (two with maximum exposure to sunlight on the southern border and two in the middle of the plot) were instrumented with a multiplexed set of specially-made thermocouples placed in different parts of the active xylem tissues. Measurements were made uninterruptedly from winter 2017 to spring 2019 at 10-min. intervals: inside small branches (1 cm diameter), inside the trunk at 2 and 4 cm depths, and in the soil underneath the trees at 20 cm depth. This set was replicated on the southern (sunlit) and northern side of each tree; *in situ* standard measurement of air temperature at 1.5 m height was also recorded. The experiment was replicated in a nearby almond tree plot. Solar radiation, air temperature and wind speed were measured concurrently in a nearby meteorological station. Our results indicate that winter standard air temperature minimums closely match those of all the above-ground xylematic systems, while maximums may differ substantially (> 20 °C), especially in the deeper xylem rings. For these parts and the root xylem, a model of heat transfer which includes the shading effect may be necessary. These results would allow a better parametrisation of epidemiological models used to estimate the development of Xf infection in woody hosts.

Acknowledgement

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Xylella fastidiosa: Risk and impact assessment

Oral presentation

Monitoring the impact of *Xylella* on Apulia's olive orchards using Sentinel-2 satellite data and aerial photographs

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**European Commission Joint Research Centre*

Abstract: Official surveillance of the *Xylella fastidiosa* (Xf) epidemic in Apulia shows how its front has moved northwards since 2013. However, assessing and modelling the impact of Xf remains difficult because little is known about the damage to the landscape behind the front lines.

Sentinel-2 and other satellite sensors provide images of the Earth in wavelengths that are sensitive to the vegetation condition. To test their suitability for Xf damage mapping, we created a model that relies on the shift that Xf-affected olive orchards undergo from a tree-dominated landscape to one dominated by shallow-rooted, short-statured vegetation. This transition changes the way gross primary productivity, as approximated by vegetation indices, responds to meteorological conditions. *In situ* estimates made by plant pathologists in olive orchards confirmed that a new method based on satellite and meteorological data detected Xf damage severity (R2 = 0.6). Furthermore, this method can be used to map Xf damage every year across Apulia.

We then tallied the individual olive trees lost since the first detection of Xf in 2013. We used aerial photographs from the summer of 2013 to delineate each olive tree in the region and photos of 2015 and 2018 to determine how many of them are now crownless, or gone altogether. We trained a Mask Region Convoluted Neural Network to delineate individual olive tree crowns in the 4-band photographs and it proved capable of accurately identifying crowns, irrespective of their size, shapes, proximity to neighbouring trees, or image resolution.

Our results provide a unique view on the spread of Xf in Apulia over the past five years, a means of systematically monitoring Xf damage across orchards, and an estimate of the number of olive trees lost thus far. It illustrates how remote sensing can provide a quantitative baseline for addressing the environmental and economic damage of Xf.

Oral presentation

Potential impact of *Xylella fastidiosa* subsp. *pauca* in European olives : A bio-economic analysis

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Abstract: *Xylella fastidiosa* is the causal agent of plant diseases which cause massive economic damage (Almeida, 2016; Chatterjee et al., 2008). In 2013, a strain of *X. fastidiosa* subsp. *pauca* was for the first time detected in Italian olives (European Food Safety Authority, 2015; Saponari et al., 2016). Here, we simulate future spread of the bacteria based on climatic suitability modelling and an assumption of radial range expansion. An economic model computes impacts by accounting for discounted foregone profits and losses in investment. The model computes impacts for Italy, Greece and Spain as these countries account for around 95 per cent of the European production (Eurostat, 2016). Climatic suitability modelling indicates that, depending on the suitability threshold, 92.5 to 95.4, 88.6 to 89.5 and 85.8 to 98.5 per cent of the national areas of production fall into suitable territory in Italy, Greece and Spain, respectively. Across the elicited rates of radial range expansion (Bragard et al., 2019), the potential economic impact over 50 years ranges from 3.58 to 8.69 billion euro if replanting with resistant varieties is not feasible. If replanting is feasible, the impact ranges from 2.00 to 4.13 billion euro. Depending on whether or not replanting is feasible, between 0.67 and 1.64 billion euro can be saved over the course of 50 years if the spread is reduced from 5.18 km to 1.1 km per year (50% and 5% percentile of elicited spread rate). The analysis highlights the major economic benefits of replanting with resistant olive cultivars and spread control. This stresses the necessity of strengthening the ongoing research on resistance traits and vector control.

Oral presentation

Empirical assessment of regional vulnerability to *Xylella fastidiosa* based on environmental factors

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**CIHEAM – CHANIA – Mediterranean Agronomic Institute of Chania (GR)*

Abstract: *Xylella fastidiosa* has already been recorded in numerous areas around Europe. Once the disease has been introduced in an area, its spread can depend on a number of factors. The biology of the vector, as well as the bacterium itself, in conjunction with the environmental factors such as climate, topography, soil characteristics and land cover will affect the rate of expansion, if left unchecked. This study combines such environmental data from some of the case studies involved in the XF-ACTORS project, with positive identification of *X. fastidiosa*, in order to empirically identify the optimum combinations of values of those parameters. This method assumes no intervention from the authorities or the stakeholders that could prevent the spreading and appearance of the disease in uninfected areas. In addition to the environmental factors, the spatial distance to the nearest positive identification of the disease is also taken into consideration. Subsequently, a vulnerability scale is generated, with areas exhibiting similar combinations of values being classified as highly vulnerable to the bacterium. This scale is applied to the entirety of the regions studied, in an effort to offer a small indication of the likelihood of the disease appearing in currently disease-free areas, where preventive action is mostly needed.

POSTER

Media representations of *Xyllela fastidiosa* progress and effects – a cognitive–semantic analysis

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Abstract: The imminent threat of *Xyllela fastidiosa* has spurred a lot of concern in the affected countries, as well as those where it might yet occur. Here, we analysed the language used to talk about the bacterium using the theoretical framework of the conceptual metaphor theory and critical discourse analysis. More precisely, we aim to discover how *X. fastidiosa*, its spread and effects are framed in public discourse and what the possible effects are of such framing for understanding and controlling the bacterium. The corpus for the analysis comprised 25 texts (totalling 15,153 words) from newspaper and internet sources in English which reported on the bacterium's outbreaks, progress and containment actions in the period from 2015 to 2019. The results indicate that metaphoric conceptualisation focuses on several aspects. First of all, the discourse about *X. fastidiosa* reveals that the bacterium is conceptualised as an entity which is deadly (deadly, lethal, devastating), ominous (it looms, haunts olive oil growers), unpredictable (an unpredictable menace) and unprecedented (an unprecedented disease, a game changing pest, a game changer) or as some other disease (olive tree leprosy, the Ebola of olive trees). Its progress is structured in terms of a journey (it crosses over borders, it reaches the shores, arrives, hitchhikes, it is on a devastating path). Its effects are structured in terms of destruction (it destroys, devastates, wipes out vineyards and olive groves, it wreaks havoc) and killing (it strikes trees, it attacks, it kills, it strangles plant tissues, plants fall victims to it). It is also conceptualised as an enemy (it marches on, it invades the plants) and hence needs to be fought, destroyed, fended off, combated and eradicated. It may be concluded that the most pervasive metaphorical images in media representations related to the bacterium's spread and effects serve to raise awareness of the gravity and magnitude of its occurrence, as well as justify the severe measures undertaken to fight it.

Poster

The Digital Research Object Portal on *Xylella fastidiosa*

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**European and Mediterranean Plant Protection Organisation (EPPO)*

Abstract: Opening research data and publishing open access articles have become requirements for many publicly funded research projects. This is the case for research projects funded through EU Framework Programmes. The number of institutions that host open research data has increased to adapt to different types of data, and scientific journals are adapting to the open access policy. The multiplication of open data/open access infrastructure has to be seen positively as it contributes to the visibility, use and re-use of scientific information. Yet, the wide diversity of such infrastructure has the counter-effect of scattering information which may be more difficult to reach.

An online publicly accessible portal (Digital Research Object Portal, DROP) has been built as part of the XF-ACTORS project. The Digital Research Object Portal works as a node, a unique entry point that facilitates access to digital research objects (data and documents) on *Xylella fastidiosa* stored on different platforms. Retrieval of data and documents is possible through the use of keywords that describe the content of the digital objects.

Data and documents produced as part of the XF-ACTORS project will be referenced in the next months. These include: genomic data, mass spectra profile data, geospatial datasets, airborne image data, eco-physiological data, open access scientific articles, guidelines, protocols and pictures.

Data and documents produced as part of other initiatives, such as the EU-funded project POnTE, the COST Action EuroXanth, the Euphresco project PROMODE and a number of national projects, will be referenced.

If successful, the portal will be maintained after the end of the project by Euphresco, the network for phytosanitary research coordination and funding and its scope will gradually expand to cover all regulated and emerging pests.

Poster

Environmental risk assessment of *Xylella fastidiosa* subsp. *pauca* in Apulia, based on ecosystem services

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Abstract: The entry, spread and establishment of *Xylella fastidiosa* in the olive-producing agroecosystem of Apulia (Italy) affect the flow of ecosystem services and biodiversity in the region. Besides the direct impacts of this invasive pest, control measures against it also have an environmental impact (e.g. the impact of continuous removal of vegetation or use of insecticides and pesticides on biodiversity; and uprooting of centuries-old trees on cultural heritage). The objective of this study is to assess the short- and long-term negative impact of *X. fastidiosa* subsp. *pauca* invasion and the control measures against it on the flow of ecosystem services and biodiversity in the olive-producing agroecosystem of Apulia at landscape level, by considering the olive groves and the embedding landscape, which includes other landscape elements than olive groves. Based on the guidance from the European Food Safety Authority (EFSA PLH Panel, 2014) for conducting environmental risk assessments of invasive pests based on ecosystem services, we conducted expert knowledge elicitation: (i) to identify the affected ecosystem services and the associated service-providing units (e.g. species, functional groups, communities, ecosystems) that are responsible for the production and regulation of these services, and (ii) to quantify (in terms of percentage change) the reductions in affected ecosystem services and the impacts on biodiversity components. Results of the elicitation will be presented.

Xylella fastidiosa: Sustainable control measures

Oral presentation

The VSPP, a voluntary certification programme to produce healthier plants for planting in the EU

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**European and Mediterranean Plant Protection Organisation (EPPO)*

Abstract: The Voluntary System Preventing Pests (VSPP) was developed within Task 9.4 of the XF-Actors project by a group composed of experts from national scientific organisations, industry, national plant protection organisations and international organisations. It aims at better integrating plant health risks in the production of plants for planting. This voluntary certification system is firstly based on the implementation in companies of quality management processes. Certified companies must also apply general plant health requirements for the prevention of pests, including the identification of critical points in the production chain. In addition to these general requirements, technical requirements for the prevention of specific pests (initially *X. fastidiosa*) can be applied for selected plant species and to a specific category of plants. These pest-specific requirements could later be extended to other pests that may represent a risk for the industry in the EU. This voluntary system builds upon the current EU and national regulations and proposes additional requirements for EU *X. fastidiosa*-demarcated areas (e.g. analysis of critical points) as well as for the rest of the territory (e.g. initial testing for all host plants and production at insect-proof facilities for the whole production chain). General rules of the VSPP have been developed taking into account the minimum requirements for a pest risk management plan as described in Article 91 of Regulation (EU) 2016/2031. Therefore, any participant certified according to the VSPP certification programme will be regularly audited, which could facilitate the approval of its pest risk management plan by the national plant protection organisations. The participant may then be subject to plant passport inspections with a reduced frequency. A first draft of a Standard has been finalised and is now shared more widely with other stakeholders. The possible organisation of the system is also being discussed.

Oral presentation

N-acetyl-cysteine for controlling *Xylella fastidiosa* in citrus and olive: understanding the differences to improve management

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Abstract: A new approach to control citrus variegated chlorosis (CVC) caused by *Xylella fastidiosa* is the use of N-acetyl-cysteine (NAC). CVC symptoms include reduction in fruit size making it not useful for juice or fresh fruit consumption. To analyse the effect of NAC under field conditions, severe CVC symptomatic plants were treated with NAC during two season harvests (2013–2014 and 2014–2015). A significant increase in fruit diameter was observed in the two seasons in plants treated with NAC-fertiliser, improving its quality for commercialisation. A new field trial was carried out during seasons 2017–2018 and 2018–2019 with similar results to those observed before. Based on the results of NAC on CVC, some field trials were performed in southern Italy to verify its effect on olive quick decline syndrome (OQDS), a novel olive disease caused by a highly pathogenic strain of *X. fastidiosa* subsp. *pauca*. The assessment was made based on the level of symptoms on branches (dieback and dessication) of olive trees selected from plots of a highly susceptible cultivar. In general, treatment with NAC seems to decrease the disease progression, especially using NAC-endotherapy. These results were more evident in the trials set up on the plots with low initial incidence of symptomatic trees, where for three years (from 2016 to 2018) some differences were recorded. But, in 2019 as the pressure of inoculum increases in the environment, an increase in disease progression was observed in all treatments. Taken together, as also observed for CVC, NAC does not completely control the bacterium, but does interfere with the progression of the disease. However, in CVC-treated plants, NAC leads to an improvement in fitness of diseased plants during the time of treatment, which was not observed for olive. Some considerations must be pointed out when comparing CVC and OQDS pathosystems. The disease in olive (and in particular in the highly susceptible cultivar used in our experiments) is more severe than in sweet orange, for which no plant death is observed. This suggests that citrus may have a more efficient plant defence response that, along with the effect of NAC, may improve disease resistance. Similarly, it is worth extending the NAC applications to infected olives of less susceptible/resistant cultivars, in the attempt to strengthen the innate host response with the positive effect of the NAC application. In addition, in commercial citrus orchards the control of vectors helps to prevent new infections and consequently the use of NAC may be more effective.

Oral presentation

Further acquisition on the response of a large number of olive cultivars to infections caused by *Xylella fastidiosa* subsp. *pauca*, ST53

Saponari M, Altamura G, Abou Kubaa R, Montilon V, Saldarelli P, Specchia F, Palmisano F, Silletti MR, Pollastro P, Zicca S, Roseti V, Manco L, Boscia D*

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Abstract: Deployment of resistant varieties is a key strategy for mitigating economic losses due to *Xylella fastidiosa* infections in crops, particularly in those areas where the bacterium finds favourable conditions for its persistence and infections are associated with severe diseases. As such, genetic traits that confer resistance/tolerance have been identified in the past in grapes, citrus and, more recently, in olives. Although understanding the molecular pathways involved in the resistance is a primary target of many studies, nowadays supported by the powerful resolution of the high-throughput sequencing technologies, biological confirmation remains a paramount tool to ultimately define the response of a given plant genotype. In this context, the emergence of *X. fastidiosa* in olives in southern Italy, prompted for large surveys and biological screening tests of olive germplasm in open fields and in greenhouses. Test plants are monitored for symptoms, incidence of bacterial infections, estimation of the bacterial population size and, more recently, analysed for the expression of LRR-RLKs- and ABA-related genes. The results so far collected highlighted the following aspects: (i) under controlled conditions the latent period of the infections in olives is as short as one year in the most susceptible cultivars, whereas under field conditions a first reliable estimation of the symptoms associated with the infections could be retrieved upon at least three years of exposure to the natural pressure of inoculum; (ii) cultivars under testing could be firstly distinguished as 'highly susceptible' those that developed symptoms in the shortest time and those 'potentially tolerant/resistant' deserving further investigation; (iii) upon needle-inoculations or field exposure, cultivars could be differentiated based on the rate of systemic infections, as highly infected vs low-infected. Interestingly, several cultivars either in greenhouses or in field testing produced results similar to those recorded for the cultivar Leccino, used as a resistant control; indeed cultivars harbouring high bacterial titers but not showing symptoms (i.e. with traits of tolerance) have also been identified. Although preliminary, these studies provide information on the spectrum of tolerance/resistance of additional olive cultivars.

Acknowledgement

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Oral presentation

Strategies for reducing vector populations and transmission of *Xylella fastidiosa* in olive groves

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Abstract: Much of the work regarding insecticidal efficacy against *Philaenus spumarius* has been initiated only in the past few years, when the control of this spittlebug species in the area where outbreaks of *Xylella fastidiosa* emerged, became essential. Among the numerous formulations (synthetic and organic insecticides) tested to control the adults on olives, a major affected European crop, neonicotinoids and pyrethoids showed the highest efficacy and persistence (Dongiovanni et al., 2018). These trials were extended in 2018 and 2019 by testing a new formulate based on acetamiprid and one based on cyantraniliprole, a systemic insecticide belonging to anthranilic diamide. For both, results showed high efficacy against *P. spumarius* indicating they could be adopted for controlling spittlebug populations in the context of the application of containment measures for *X. fastidiosa*.

With the purpose of reducing the efficiency of *X. fastidiosa* vector transmission under organic farming management, applications of kaolin were tested for four consecutive years as a preventive approach to protect a new olive plantation exposed to the natural inoculum pressure. However, applied on a calendar basis, the use of kaolin did not protect the young olives from infections and subsequent symptoms development. Surprisingly, this was also the case for the plants treated with the insecticide used as controls, based on imidachloprid, for which, even if to a lower extent, infections also occurred.

Attempts were also made to implement strategies, alternative to the mechanical control of weeds, for controlling the juveniles, a stage of the insect life when they are more vulnerable and control can be more efficient. Sowing different gramineous species to replace the natural ground vegetation, applications of herbicides and pyroherbicides, were compared. Soil tillage, pyroherbicides and herbicides applied in spring were the only interventions able to reduce almost to zero the presence of juvenile spittlebugs.

The experimental data herein developed will be helpful for the end-users to choose better options for the management of this vector in different agroecosystems.

Oral presentation

Understanding the olive microbiome of susceptible and resistant cultivars for sustainable biocontrol

Baptista P*, Cameirão C, Giampetruzzi A, Morelli M, Abou Kubaa R, Altamura G, D'Attoma G, Pereira JA, Lino Neto T, Sisto A, De Bellis P, Saldarelli P

**Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Bragança (PT)*

Abstract: Olive quick decline syndrome (OQDS) is a devastating olive disease, which emerged few years ago in the region of Apulia (southern Italy) as a result of the bacterial infections caused by *Xylella fastidiosa* subsp. *pauca*. Bacterial infections were consistently associated with severe desiccations on the local cvs Cellina di Nardò and Ogliarola salentina, whereas mild symptoms were found in the infected trees of the cvs Leccino and FS17, indicating that these cvs may harbour traits of resistance. Investigations on the olive microbiome of OQDS-resistant and susceptible cultivars were undertaken to identify potential protecting endophytes for a sustainable strategy of biocontrol. In this study, bacterial and fungal communities of the xylem of infected trees of the cvs FS17 and Kalamata, respectively symptomless and highly symptomatic, were analysed by barcode (16 S rRNA V4 and ITS1-spanning amplicons) and whole shotgun (WSS) sequencing. Overall, the core microbiome was dominated by fungi, accounting 99.8% and 88.4% of the total reads by barcode and WSS sequencing, respectively, while Proteobacteria and Ascomycota are the most represented phyla with both techniques. This fungi/bacteria ratio was maintained in all trees of the cv FS17 while it was inverted in the susceptible cv Kalamata, in which *Xylella* colonised the majority of the ecological niche in the heavily infected plants. Bacteria were isolated from the sapwood of olive trees of the cv Kalamata and FS17 and plate assays were performed to evaluate the antagonistic activity of these olive endophytes against *X. fastidiosa*. Moreover, bacterial communities of selected trees of the cv FS17 were isolated, purified and co-inoculated with *X. fastidiosa* into potted olive plants of the cv Cellina di Nardò for evaluating potential effects on the evolution and progression of *Xylella* infections in this susceptible cultivar. Data on antagonistic activity of isolated endophytes and the progress of *Xylella* infections and/or symptom appearance in these microbiome–Xf co-inoculated plants will be presented.

Acknowledgement

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

Oral presentation

Assessment of *Paraburkholderia phytorfirmans* PsJN biocontrol potential against *Xylella fastidiosa* 'De Donno' strain in olive

Morelli M*, Dongiovanni C, D'Attoma G, Giampetruzzi A, Loconsole G, Montilon V, Altamura G, Angione D, Saponari M, Saldarelli P

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

Abstract: Numerous attempts have been made to test the use of endophytic bacteria to control diseases caused by *Xylella fastidiosa* (Xf), but promising results obtained *in vitro* have often proved inadequate once transferred to the field.

A new encouraging possibility came from the observation that the bacterium *Paraburkholderia phytofirmans* PsJN, widely studied for its ability to defend plants from biotic and abiotic stresses, was able to reduce the symptoms caused by Xf in grapes affected by Pierce's disease.

Our study aimed at testing the effectiveness of PsJN as a biocontrol agent in the Xf strain 'De Donno'/olive pathosystem. Although *in vitro* tests showed the absence of competitive inhibitory effects of PsJN on Xf 'De Donno' growth or its ability to form biofilm, several trials were started both in greenhouse and in Xf-contaminated orchards. Although different approaches were tested to deliver PsJN in olives, high rates of successful isolations and positive detection, achieved with a SYBR? -Green-based qPCR assay ad hoc developed in this study, were obtained only upon needle inoculation of 1- to 2-year-old shoots, in which PsJN proved to remain viable for >500 days.

Current observations in open-field trials, so far limited to a single season, have not revealed significant differences in the reduction of OQDS symptoms or Xf concentration in therapeutic treatments, between plants treated or not with PsJN, nor reduction of the new infections upon preventive applications. Despite the evidence that PsJN can colonise the xylem vessels, time course diagnostic tests clearly showed that it moves slowly away from the point of inoculation and its concentration decreases significantly over time.

This absence of systemic colonisation suggests a possible induced response of the plant, which will need to be analysed in detail, to optimise treatments and trigger a cascading effect on Xf. In this direction, preliminary results of PsJN impact, on the resident microbiome diversity indices, in presence/absence of Xf, have been gathered using a WGS approach.

Acknowledgement

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

Oral presentation

Monitoring viable cells by means of PEMAX-qPCR for screening and evaluation *in planta* of bactericidal compounds against *Xylella fastidiosa*

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Abstract: Currently there are no efficient methods to cure *Xylella fastidiosa*-infected plants, therefore methods to monitor the pathogen in plant hosts during the asymptomatic stage of the disease are necessary. Our research deals with the development and evaluation of antimicrobial and defence elicitor peptides against *X. fastidiosa*, and with the analysis of aggressiveness of isolates from different origins in model plant hosts of economic importance. In these studies it is required to know about the physiological state of the pathogen, either in the infected plant or upon interaction with the antimicrobials.

It is well known that the *in vitro* culture of this bacterium presents difficulties due to its low cultivability in synthetic media. Long-term incubation periods (up to six weeks) are required to observe growth. Besides this, and because it is a quarantine pathogen in Europe, strict security measures for working with this bacterium have been imposed for avoiding the risk of spread. Thus, manipulation of cultures and infected plants has to be reduced to a minimum.

In this context, there is a need to develop and optimise molecular methods to detect and quantify viable *X. fastidiosa* cells, as an alternative/complement to plate counting and without the necessity of manipulating cultures or infected plant samples. The viable quantitative PCR (v-qPCR) is a methodology already developed for the quantification of viable foodborne pathogenic microorganisms in different food matrices and for performing viability assessment of biological control strains in the field. This technique constitutes a method that allows the quantification of only viable cells by using nucleic acid-binding dyes such as propidium monoazide (PMA) or ethidium monoazide (EMA) in combination with qPCR. In both cases, the dye intercalates between the DNA of the compromised or non-metabolically active cells, avoiding its amplification afterwards by qPCR.

This methodology that makes it possible to determine viable cells, when combined with conventional qPCR (total *X. fastidiosa* cells), and plate counting (culturable cells), provides information on the physiological states of the bacteria. Thus, it is an advantageous technique for evaluating bactericidal compounds, as well as monitoring the colonisation of *X. fastidiosa* in different host plants during the disease progression.

Poster

Field testing of antimicrobial compounds to mitigate *Xylella fastidiosa* infections in olives

Dongiovanni C*, Di Carolo M, Fumarola G, Palmisano F, Zicca S, Silletti MR, Perrelli D, Boscia D, de Souza A, Saldarelli P, Saponari M

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Abstract: The severe impact of *Xylella fastidiosa* subsp. *pauca*, ST53, infections in olives prompted an intense programme of field testing of different formulations for curative purposes. Under our experimental test conditions several compounds (Fosetyl alluminium; Protein of Harpin; COS-OGA; Acibenzolar S-methyl) proved to be inefficacious to reduce the occurrence and the severity of the desiccation phenomena induced by *X. fastidiosa* on the susceptible olive cultivars. However, among these, applications of N-acetylcysteine (NAC) known to disrupt the biofilm matrix of *Xylella* cell aggregates, and ammonium chloride provided some encouraging attenuation of the symptoms. Briefly, NAC was tested in the field in four different trials under different experimental conditions (trees of different ages, with different initial incidence of symptoms and infections) and modes of application (fertirrigation, soil application mixed with organic fertilisers, trunk injections). Ammonium chloride was initially applied by a local grower by spraying the olive canopies in an attempt to save the olive trees, the evident reduction of symptoms observed prompted then in spring 2019 to set *ad hoc* trials to test different concentrations and number of applications. For NAC, endotherapy applications (one per year) in new plantations (preventive treatments) or in olive groves with only limited initial incidence of the infections, were the only conditions that yielded some reduction in the occurrence of dieback and branch desiccation, even if quantitative PCR on the trees did not show any significant reduction (treated vs non-treated controls) in the bacterial population size. Regardless of the mode of application, the uptake of NAC was confirmed in all cases by HPLC analysis and by the phytotoxicity effects (leaf drop) recorded when the highest doses were used. Similarly, for the olive trees sprayed with ammonium chloride, even if clear symptom reduction was recorded, no differences were recorded on the bacterial populations in the mature tissues of the plants. Along with further observation and tests, the competence of the bacterium to colonise the new growth and its vector-transmissibility from the treated trees (NAC and ammonium chloride) will be assessed.

Poster

Searching for *Xylella fastidiosa* solutions: survey natural enemies of *Auchenorrhyncha* eggs

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Abstract: The dangerous phytopathogenic bacteria *Xylella fastidiosa* (Wells et al., 1987) has been recently detected in Portugal (January 2019). This disease is transmitted horizontally from infected to non-infected plants by xylem-feeders that belong to the suborder Auchenorrhyncha. The information available until now about natural enemies is reduced. In this work the potential natural enemies of Auchenorrhyncha eggs, the most susceptible stage, were studied during the autumn/winter of 2018/2019. For that, from November 2018 to February 2019 before the egg hatching, on a biweekly basis, 10 samples of 50 g of rests of the remaining vegetation in the ground cover, mainly Poaceae, were collected in an olive grove. In the laboratory, leaves, particularly the interior part of the blade which is a common oviposition location for spittlebugs, were observed under binocular stereoscope. Viable eggs were introduced in petri dishes until hatching. A total of 647 egg-laying masses and 8,222 eggs with a mean of 12.7 eggs per egg laying was recorded. Egg masses were characterised and the action of predation, parasitism and fungi were recorded. Eggs presenting signs of parasitism plus predation were more than 50%. Intact field eggs were kept in controlled conditions until hatching of nymphs and evolution. The parasitoids were identified as *Paracentrobia* sp. (Trichogrammatidae) being the first report for the genus in Portugal. These results constitute an important opportunity to control the main insect vectors of *X. fastidiosa* and containing its spread.

Acknowledgement

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Poster

Methylobacterium spp., endophytes of olive trees, as potential biocontrol agents of *Xylella fastidiosa* subsp. *pauca*

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Abstract: Interactions between endophytes and plants can promote the health of the host and play a significant role in low-input sustainable agriculture. Understanding this plant–microbe interaction and the mechanisms that enable endophytes to enhance the plant defence response is essential, especially for endophytic bacteria that show biocontrol potential against vascular wilt pathogens. Investigations on the bacterial endophytic population occurring in the xylem of healthy and *Xylella fastidiosa* subsp. *pauca* ST53 (XfpST53)-infected olive trees showed that under field conditions, the population level of cultivable endophytic bacteria is highly variable, being mainly affected by the host genotype, host age, and wilting severity. Among the different cultivable bacteria occurring in the wood of olive trees, *Methylobacterium* spp. are one of the most interesting groups. *Methylobacterium* strains isolated from the xylem of healthy and XfpST53-infected olive trees have been identified as *M. mesophilicum* and *M. radiotolerans*. Species of *Methylobacterium* have also been reported as potential biocontrol agents, plant-growth-promoting bacteria, and resistance inducers, by producing phytohormones, inducing plant systemic resistance, and supplying or mobilising nutritional elements (siderophore production). In order to evaluate the potential of *M. mesophilicum* GR19, and *M. radiotolerans* GR18, GR22 e GR23, as nutrient competitors of XfpST53, the production of siderophores was investigated by using the Chrome Azurol S (CAS) agar and ferric perchlorate assay to detect hydroxamates. *M. mesophilicum* DSM 1708 and *M. radiotolerans* DSM 1819 were used as reference strains. All the tested strains produced different levels of siderophores, and the most effective were applied by endotherapy in healthy and XfpST53-infected olive trees, in order to evaluate *in planta* their activity in containing the olive quick decline syndrome. Moreover, the characterisation of plant-growth-promoting traits of several *Methylobacterium* strains are currently in progress, i.e. by screening the production of indole-3-acetic acid (IAA), and the 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity.

INDEX OF PRESENTING AUTHORS

The Projects in brief

The POnTE Project
Grant Agreement 635646

TOPICS

POnTE focuses its activities on the investigation of genetics, biology, epidemiology, vector ecology and economic impacts of three main pathosystems that threaten strategic crops and natural landscapes in the EU

***XYLELLA FASTIDIOSA* (*XF*) AND HEMIPTERAN VECTOR SPECIES**. The harmful bacterium *Xf* is involved in a new and severe olive disease (Olive Quick Decline Syndrome – OQDS) firstly reported in 2013 in southern Italy (Apulia region, Salento peninsula). Preliminary investigations showed that symptomatic olive trees were affected by a biocomplex of pests and plant pathogens: the Gram-negative bacterium *Xf*, several pathogenic fungal species and Zeuzera pyrina (Leopard moth). *Xf* was previously isolated from olive in California, whose strain proved to be phylogenetically related to subsp. *multiplex*, and classified as "Genotype A". In contrast, *Xf* isolated from OQDS in Italy was identified as a novel and distinct genotype (denoted as "CoDiRO *Xf* strain"), which has a phylogenetic relationship with isolates of *Xf* subsp. *pauca*. In February 2014, a *Xf* strain with a genetic profile similar to the CoDiRO *Xf* was found and identified in oleander in Costa Rica. Although *Xf* is widely distributed and studied in the Americas due to diseases caused in grapevine, fruit trees, and landscape plants, the recent outbreak of *Xf* in olive trees in southern Italy is the first confirmed presence of *Xf* in the EU. *Xf* is exclusively transmitted by xylem-fluid feeding insects. A preliminary survey of the hemipteran population in *Xf* foci area indicated that the primary xylem-feeding insect there was the spittlebug *Philaenus spumarius*. PCR assays of head capsules of P. spumarius collected from weeds in olive groves with OQDS in this area showed that a high percent was positive for *Xf* and transmission tests proved P. spumarius main role in the Xf CoDiRO strain transmission.

'*CANDIDATUS* LIBERIBACTER SOLANACEARUM' AND PSYLLID VECTOR SPECIES. *Ca*Lsol is a recently described phloem-limited, Gram-negative, not culturable bacterium that has emerged as one of the most important pathogens affecting potato and other solanaceous crops (i.e. tomato and pepper) in the Americas and New Zealand. Recently EPPO has recommended member countries to regulate solanaceous haplotypes of *Ca*Lsol and its psyllid vector Bactericera cockerelli as quarantine pests, since non-solanaceous *Ca*Lsol haplotypes have now been found in Europe associated with diseased carrots and celery. The emergence of these *Ca*Lsol haplotypes in carrots and celery has raised serious concerns about the risk that they pose to potato and other solanaceous crops across the whole EU.

***HYMENOSCYPHUS FRAXINEUS* (ANAMORPH. CHALARA FRAXINEA) AND NEW AND EXOTIC PHYTOPHTHORA SPECIES**. Hp is a pathogen introduced, for the first time in Poland in 2006, via plant trade, mainly affecting common ash (*Fraxinus excelsior*) and the narrow-leafed ash (*F. angustifolia*). The disease is usually fatal and has now been reported in most continental European countries as a very serious threat to ash populations. In addition, an increasing number of new emerging diseases affecting forest trees caused by several *Phytophthora* spp. are leading to significant economic losses and pose considerable risks to natural ecosystems. The knowledge of the genus *Phytophthora* is still limited and some hybrid species are still evolving, potentially increasing the risk of colonization of new forest hosts.

SPECIFIC OBJECTIVES

The specific objectives of POnTE are focused on the investigation of genetics, biology, epidemiology, vector ecology and economic impacts of four pathosystems that threaten strategic crops and natural landscapes in the EU in order to identify economically, technically feasible and environmental sustainable integrated management strategies for the containment of each pathosystem. For each target, the research activities will implement the state-of-the-art and provide a novel scientific background to sustain future management policies. The specific objectives will broadly cover all targeted pathosystems merging multidisciplinary research with the practical needs of the stakeholders and end-users.

LIST OF BENEFICIARIES

P1	CNR, ITALIAN NATIONAL RESEARCH COUNCIL, Italy
P2	UNIBA, UNIVERSITY OF BARI ALDO MORO, Italy
P3	INRA, FRENCH NATIONAL INSTITUTE FOR AGRICULTURAL RESEARCH, France
P4	ANSES, FRENCH AGENCY FOR FOOD, ENVIRONMENTAL AND OCCUPATIONAL HEALTH AND SAFETY, France
P5	IVIA, VALENCIAN INSTITUTE FOR AGRICULTURAL RESEARCH, Spain
P6	CSIC, SPANISH NATIONAL RESEARCH COUNCIL, Spain
P7	SG SASA, SCOTTISH GOVERNMENT–SCIENCE AND ADVICE FOR SCOTTISH AGRICULTURE, United Kingdom
P8	FORESTRY COMMISSION RESEARCH AGENCY, United Kingdom
P9	BFW, FEDERAL RESEARCH AND TRAINING CENTRE FOR FORESTS, NATURAL HAZARDS AND LANDSCAPE, Austria
P10	LUKE, NATURAL RESOURCES INSTITUTE FINLAND, Finland
P11	WU, WAGENINGEN UNIVERSITY, The Netherlands
P12	NIBIO, NORWEGIAN INSTITUTE OF BIOECONOMY RESEARCH, Norway
P13	UCR, UNIVERSITY OF COSTA RICA, Costa Rica
P14	ARO, AGRICULTURAL RESEARCH ORGANIZATION OF ISRAEL, THE VOLCANI, Israel
P15	UB, UNIVERSITY OF BELGRADE, Serbia
P16	CERTIS EUROPE B.V., The Netherlands
P17	AUREA IMAGING BVBA, Belgium
P18	VILMORIN S.A., France
P19	LOEWE BIOCHEMICA GMBH, Germany
P20	PHYTOPHTHORA RESEARCH AND CONSULTANCY, Germany
P21	ACLI RACALE–AGRICULTURAL COOPERATIVE SOCIETY, Italy
P22	AGRITEST SRL, Italy
P23	CITOLIVA FOUNDATION, INNOVATION AND TECHNOLOGY CENTER FOR OLIVE FARMING AND OLIVE OIL, Spain
P24	AGRICULTURAL VILLENA COOPERATIVE, Spain
P25	A L TOZER LTD, United Kingdom

IN KIND CONTRIBUTIONS

P1 DEPARTMENT OF AGRICULTURAL, FOREST AND FOOD SCIENCES (DISAFA) OF THE UNIVERSITY OF TORINO, Italy

P2 CENTRO DI RICERCA, FORMAZIONE E SPERIMENTAZIONE IN AGRICOLTURA (CRSFA) “BASILE CARAMIA”, Italy

P10 DEPARTMENT OF AGRICULTURAL SCIENCES OF THE UNIVERSITY OF HELSINKI, Finland

CONSORTIUM BODIES

THE COORDINATOR

His primary role is to represent the intermediary between the European Commission (EC) and the Consortium as well as to be the promoter and supervisor of the overall technical and scientific progress of POnTE.

Donato BOSCIA **(CNR-IPSP, Italy)**

THE SCIENTIFIC COORDINATION TEAM

The Scientific Coordination Team is a management body chaired by the Project Coordinator; it is composed by the sub-Coordinators in charge for the coordination of the research and dissemination activities related to the three pathosystems targeted by POnTE.

TOPIC *XYLELLA FASTIDIOSA*

Maria SAPONARI **(CNR-IPSP, Italy)**

TOPIC *CANDIDATUS* LIBERIBACTER SOLANACEARUM

Anne NISSINEN **(LUKE, Finland)**

Françoise POLIAKOFF **(ANSES-LSV, France)**

TOPIC EMERGING DISEASES OF FORESTS

Ana PEREZ-SIERRA **(FORESTRY RES AG, United Kingdom)**

THE GENERAL ASSEMBLY

The General Assembly is the decision-making body of the Project. All Project partners are seated in the General Assembly, chaired by the Project Coordinator. The General Assembly meets annually, unless the interest of the Project may require intermediate meetings, to consider the reports of the Project Coordinator, the Scientific Coordination Team, accounts for the past financial year, and to decide upon changes to the Implementation Plan.

THE MANAGEMENT BOARD

The Management Board is the decision-implementing body of the Project. Chaired by the Coordinator, the Management Board is composed of the following persons (WP leaders), each of them holding both scientific excellence and strong experience in large collaborative research and development projects.

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THE COORDINATION TEAM

The Coordination Team, provided by CNR-IPSP, is made up by the Project Coordinator assisted by a sub-coordinator and 2 personnel Units, one from administrative and one from research staff. The Coordination Team is in particular responsible for Project administration, consolidation of the annual Project reports, financial monitoring, management of the web resources and partner assistance.

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THE SCIENTIFIC ADVISORY GROUP

The Scientific Advisory Group consists of internationally acknowledged scientists and experts from outside the Project. The main role of the Scientific Advisory Group is to provide the Project with points of view and advice coming from other scientific communities and areas interested in the outcomes of the Project.

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THE BOARD OF STAKEHOLDERS

The Board of Stakeholders is appointed from International and National Plant Protection and Quarantine services, from Policy makers at the EU level, growers, producers and nurserymen organizations and will ensure that the Consortium takes into account the interests of the stakeholders and end-users and operates for the benefit of the EU growers and of the Plant Protection Services.

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The XF-ACTORS Project Grant Agreement 727987

TOPICS

XF-ACTORS research workplan covers different topics related to *Xylella fastidiosa*:

Biology, genetics and pathogenicity

Ecology and control of vectors

Surveillance programs: tools for early detection and remote sensing approaches

Epidemiology and Pest risk assessment

Innovative and sustainable strategies for the control of *Xylella*-induced diseases

Improving capacity building and Plant health management

BIOLOGY, GENETICS AND PATHOGENICITY. *Xylella fastidiosa* (*Xf*) is a xylem-limited, plant-pathogenic bacterium with a wide host range. Processes leading to plant colonization and the specific mechanisms leading to disease and the mechanisms of host plant specificity are still poorly understood.

Thus, there is high uncertainty with regard to the potential host range of *Xf* in the European flora as a wide range of European wild plant species have never been exposed to the bacterium and it is not known whether they would be hosts, and, if so, whether they would develop symptoms.

To this end, the importance of experimental work to determine the host range of pathogens remains paramount to demonstrate that individual genotypes are pathogenic to specific host plant species. Large dataset of genomic sequences will be cooperatively developed within this project, to gather critical elements to understand the evolution of the population in the contaminated areas, and to identify critical parameters involved in *Xf*-host interaction that could be used to design novel control methods.

Molecular characterization of EU *Xf* genotypes coupled with biological tests on host range and analysis of ecological parameters will be essential in the development of quarantine, containment, and control practices.

ECOLOGY AND CONTROL OF VECTORS. Vectors are required for the natural dissemination of *X. fastidiosa*. Therefore, a robust understanding of vector ecology is necessary for the development of management practices. Some aspects relevant to the emergence of Xf is that the bacterium lacks vector specificity, thus all xylem-feeder species are potential vectors and these are distributed worldwide.

Due to the relatively recent emergence of the Xf threats in Europe, there are no consolidated data on the distribution of various potential insect vectors are available in EU, with consequent uncertainties about the area where the bacterium can spread rapidly and cause serious disease outbreaks, the project work plan will investigate the biological processes involved in insect vectoring (feeding behavior, host preference, vibrational communication signals) and the use of innovative approaches (NGS analysis, microbiome and metagenomics sequencing) to produce massive information for the development of novel bio-control tools.

SURVEILLANCE PROGRAMS: TOOLS FOR EARLY DETECTION AND REMOTE SENSING APPROACHES. Surveillance and detection of *Xylella fastidiosa* through the area of potential establishment in the EU are keys for early identification of further outbreaks and a prerequisite to effective containment and control of the bacterium and its vector.

Both Xf susceptible host mapping and the early detections of *Xf* infections, have the potential to greatly improve pest spread models, which will specifically address the need to "establish more effective mechanisms and tools for risk assessment and prevention.

The development of methodologies for rapid and sensitive bacterial detection is one of the main project task, whose outcomes will support field surveys and inspections at the point of entry, and that coupled with the use of remote sensing technologies for discovering Xf-associated symptoms at the early stage aims to provide efficient and innovative tools for prevention.

EPIDEMIOLOGY AND RISK ASSESSMENT OF XF DISEASES. The epidemiology of *Xylella fastidiosa* diseases is dependent on a variety of ecological, biotic, and abiotic factors and infection dynamics are influenced by the extensive list of host plants species that can be infected, the plant-host specificity of different *Xf* genotypes, and the wide range of potential insect vectors, i.e the epidemiology of *Xylella*-diseases may change dramatically if vector species with different host plant preferences, feeding habits, and dispersal abilities are introduced.

It is also possible that ecological conditions limit the host range and/or virulence of pathogens, which may be 'released' in new environments where other vector species and host plants are present. A biologically-detailed process-based (mechanistic) spread models representing the potential expansion of an invasive species from a location of entry, based on its population dynamics and dispersal will be explored.

This approach complements the species distribution model, incorporating the temporal disease dynamics and mechanistic underpinning. The model will be an effective tool for regional risk assessment estimating the potential future spread and dispersal.

INNOVATIVE AND SUSTAINABLE STRATEGIES FOR THE CONTROL OF *XYLELLA*-INDUCED DISEASES. Successful *Xylella*-diseases management must use an integrated strategy that involves the principles of exclusion, eradication, and protection. Exclusion of the pathogen, avoidance of the pathogen, cultural practices, control of insect vectors and disease resistance are some of the control measures.

With regard to control measures, although there are some ongoing research lines, an effective control method of the pathogen applicable in the field is lacking. Control of Xf is therefore currently achieved by removing sources of inoculum, using healthy plant propagation material and controlling the vector(s).

The project ambition is then to include different actions (i) to control the pathogen and the vector developing novel tools (i.e. use of bacteriophage, antimicrobial peptides, endosymbiotic microrganisms, etc.); (ii) to enhance host defense mechanisms, (iii) to implement agricultural practices to reduce spread, and (iv) to produce pathogen-free propagating materials for the new plantations.

The overall ambition is to develop long-term sustainability of cropping systems that protect the natural biodiversity and landscape environments throughout the entire EU territory. Such control measures will be used to produce specific cultivation guidelines for pest and disease management and for low input/organic farming, taking into account the high numbers of host plants and vectors of this bacterium and the differences across production systems which may affect the effectiveness of the measures.

IMPROVING CAPACITY BUILDING AND PLANT HEALTH MANAGEMENT. An important ambition of the project is to contribute to the capacity building of the human resources involved at international and national level in the biosecurity plan.

New requirements for imports of non-EU plants have been introduced and movements of 'specified plants' (which includes the confirmed hosts of Xf in the EU and further afield) are only possible from areas in the EU where the pathogen is present if stringent conditions are met. In the general organization of the EU phytosanitary system, the national plant protection organizations of the member states are the main part of the official services for the implementation of such provisions.

This implies that the effectiveness of the quarantine program relies on (i) coordinated measures adopted by the Member States; (ii) harmonized practical guidelines for the implementation of the EU decisions;

(iii) updated information on the susceptible hosts and on the phytosanitary status of the country of origin; (iv) suitable diagnostic tools for early detection; and (v) trained personnel, at different level, capable of organizing and performing efficient surveillance programs.

LIST OF BENEFICIARIES

P1	Consiglio Nazionale delle Ricerche (CNR)
P2	Centre International de Hautes Etudes Méditerranéennes (CIHEAM)
P3	Università degli Studi di Bari Aldo Moro (UNIBA)
P4	Institut National de la Recherche Agronomique (INRA)
P5	Agencia Estatal Consejo Superior De Investigaciones Cientificas (CSIC)
P6	Instituto Valenciano de Investigaciones Agrarias (IVIA)
P7	Benaki Phytopathological Institute (BPI)
P8	Julius Kuhn-Institut Bundesforschungsinstitut fur Kulturpflanzen (JKI)
P9	Instituut voor Landbouw- en Visserijonderzoek (ILVO)
P10	The Regents of the University of California (UC)
P11	Centro de Citricultura (IAC)
P12	National Taiwan University (NTU)
P13	University of Costa Rica (UCR)
P14	Natural Environment Research Council (NERC)
P15	Instituto Politécnico de Bragança (IPB)
P16	The University of Salford (USAL)
P17	Joint Research Centre - European Commission (JRC)
P18	Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CRA)
P19	Fondazione Centro Euro–Mediterraneo sui Cambiamenti Climatici (Fondazione CMCC)
P20	Nederlandse Voedsel En Waren Autoriteit (NVWA)
P21	Instituto Andaluz de Investigaciony Formacion Agraria Pesquera Alimentaria Y de la Producion Ecologica (IFAPA)
P22	International Federation of Organic Agriculture Movements European Union Regional Group (IFOAM EU GROUP)

P23 EPPO

P24 RUSSEL IPM LTD

P25 ENBIOTECH Srl

P26 AINIA

P27 Sustainable Communication Aisbl (S-COM)

P28 Stichting Nederlandse Algemene Kwaliteit Sdienst Tuinbouw (NAKTUINBOU)

P29 CIVI-ITALIA

IN KIND CONTRIBUTIONS

P2 CIHEAM – Bari and CIHEAM - Chania

P3 CENTRO DI RICERCA, FORMAZIONE E SPERIMENTAZIONE IN AGRICOLTURA (CRSFA) “BASILE CARAMIA”, ITALY

P27 NET7 and ID Consulting

CONSORTIUM BODIES

THE COORDINATOR

Her primary role is to represent the intermediary between the European Commission (EC) and the Consortium as well as to be the promoter and supervisor of the overall technical and scientific progress of XF-ACTORS.

Maria Saponari **(CNR-IPSP, Italy)**

THE GENERAL ASSEMBLY

The General Assembly is the decision-making body of the Project. All Project partners are seated in the General Assembly, chaired by the Project Coordinator. The General Assembly meets annually, unless the interest of the Project may require intermediate meetings, to consider the reports of the Project Coordinator, the Scientific Coordination Team, accounts for the past financial year, and to decide upon changes to the Implementation Plan.

THE MANAGEMENT BOARD

The Management Board is in charge of monitoring the progress of the activities towards the overall objective of the project in order to deliver the results in due time and coherently with the budget allocation. It has the responsibility of the supervision of the research work flow, management and overseeing Project presentations and results dissemination, approve press release(s) and external communications related to the project. The Board members include all Workpackage Leaders as reported below.

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Marie-Agnes JACQUES **(INRA, France), WP2 Leader**

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Antonio VICENT	**(IVIA, Spain), WP8 Leader**
Francoise PETTER	**(EPPO, France), WP9 Leader**
Davide MENEIRO	**(S-COM, Belgium), WP10 Leader**

THE COORDINATION TEAM

The Coordination Team is responsible for Project administration, consolidation of the annual Project reports, financial monitoring, management of the financial resources and partner assistance.

Maria SAPONARI	**(CNR-IPSP, Italy), Project Coordinator**
Luciana SAVINO	**(CNR-IPSP, Italy), Administrative Staff**

THE SCIENTIFIC ADVISORY GROUP

The Scientific Advisory Group consists of internationally acknowledged scientists and experts from outside the Project. The main role of the Scientific Advisory Group is to provide the Project with points of view and advices coming from other scientific communities and areas interested in the outcomes of the Project.

Prof.	**Mariano CAMBRA**	**former IVIA, Spain**
Prof.	**Edwin CIVEROLO**	**former USDA-ARS, USA**
Prof.	**Leonardo DE LA FUENTE**	**AUBURN UNIVERSITY, USA**
Dr.	**Willem ROELOF**	**DEFRA, UK**
Dr.	**Giuseppe STANCANELLI**	**EFSA, Italy**

THE BOARD OF STAKEHOLDERS

The Board of Stakeholders is appointed from International and National Plant Protection and Quarantine services, from Policy makers at the EU level, growers, producers and nurserymen organizations and will ensure that the Consortium takes into account the interests of the stakeholders and end-users and operates for the benefit of the EU growers and of the Plant Protection Services. Efforts have been made by the Beneficiaries for an active engagement of different stakeholders (i.e. growers, Plant Health Authorities, Nurserymen). The following members have already joined the Board, but in the course of the action, other participants will be offered the opportunity to join the Board, giving priorities to those stakeholders that are directly involved in the areas most affected by the socio-economical threat posed by Xylella fastidiosa.

Ioannidou STAVROULA	**(Hellenic Republic Ministry of Rural Development, Greece)**
Maria Milagros LOPEZ	**(AESAVE – Asociación Española De Sanidad Vegetal)**
Leonardo CAPITANIO	**(ANVE – Associazione Nazionale Vivaisti Esportatori)**
Anna RUFOLO	**(CIA, Italy)**
Catarina BAIRRAO BALULA	**(COI, Italy)**
George FRANKE	**(AIPH The International Association of Horticultural Producers, UK)**

Juan Carlos ROMERO PULGARIN	**(ASAJA Córdoba, Spain)**
Carlo Francesco CESARONI	**(Ministero dell'Agricoltura e delle Politiche Agricole, Italy)**
Tina CAROPPO	**(INNOVAPUGLIA, Italy)**
Carmen CAPISCOL	**(CITOLIVA, Spain)**
Francisca PARETS AMENGUALAS	**(COPACOGECA, Belgium)**
Josep PAGES	**(European Nurserystock Association, ENA, Belgium)**
Pantaleo GRECO	**(APROL Lecce, Italy)**
Ralf KOEBNIK	**(EUROXANTH COST ACTION, France)**
Maroun EL MOUJABBER	**(CURE-XF Consortium, Italy)**

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