



INTERNATIONAL SYMPOSIUM  
ON THE EUROPEAN OUTBREAK  
OF XYLELLA FASTIDIOSA IN OLIVE  
GALLIPOLI, LOCOROTONDO, ITALY  
21-24 OCTOBER 2014

M. L. P. 2014



## **ORAL PRESENTATIONS**



**OCCURRENCE OF XYLELLA FASTIDIOSA IN APULIA.**

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A strain of *Xylella fastidiosa* subsp. *pauca* denoted CoDiRO (abbreviation from the Italian name “Completo del Disseccamento Rapido dell’Olivo”) is associated with a novel severe disease denoted “Olive Quick Decline Syndrome” (OQDS), which appeared suddenly in 2010 in Apulia (south-eastern Italy). Prior to the discovery of this outbreak (October, 2013) *X. fastidiosa* was known to be widely distributed in the Americas, where at least four different subspecies have been described and characterized. More recently, it emerged in grapevines and pear trees in Taiwan. In these areas the bacterium is the causal agent of a number of economically important diseases, i.e. Pierce’s Disease (PD) of the grapevine, leaf scorch of almond and other stone fruits, pear, oleander and coffee, Citrus variegated chlorosis (CVC), and other diseases of perennial and landscape plants. Research activities have been promptly undertaken for the characterization of the local population of the pathogen, and for understanding its epidemiology, as an essential trait for the design of a rational plan of containment. A range of susceptible hosts other than olive has been identified, which includes almond (*Prunus dulcis*), oleander (*Nerium oleander*), cherry (*Prunus avium*), myrtle-leaf milkwort (*Polygala myrtifolia*), coastal rosemary (*Westringia fruticosa*), *Acacia saligna* and *Spartium junceum*. Moreover, a monitoring program for identifying potential sources of resistance in olive has been initiated. After the discovery of the major OQDS outbreak, located in the district of Gallipoli, several new infection foci, promptly reported to the Regional Phytosanitary Service, were discovered within the whole province of Lecce; the appearance of them increased pre-occupantly during summer 2014. This spatial-temporal evolution of the epidemic in the Salento peninsula is discussed, as well as the preliminary data on the possible role in *X. fastidiosa* epidemiology of each alternative host so far identified.

**POTENTIAL VECTORS OF XYLELLA FASTIDIOSA IN EUROPE.**

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*Xylella fastidiosa* is a xylem-limited bacterium that is exclusively transmitted by xylem-sap feeding insects belonging to the order Hemiptera, sub-order Cicadomorpha. Vectors acquire the bacterium by feeding in the xylem of an infected plant and can inoculate the pathogen to healthy plants immediately after acquisition. Bacteria are restricted to the foregut and do not systemically infect the insect body, therefore vectors lose the infectivity after moulting. However, once infected, adults transmit persistently for life, because the bacterium multiplies and persists in the vector foregut. As for transmission specificity, although *X. fastidiosa* transmission is restricted to xylem-sap feeding insects, there is no species-specificity and all xylem-sap feeding insects are considered potential vectors. Following the recent introduction of *X. fastidiosa* in the Salento area of Italy, a thorough analysis of the potential European vector species was undertaken. The results underline a striking difference in the fauna of xylem-sap feeding insects between the New World and Europe. In particular, while in the Americas there are numerous sharpshooters species (family Cicadellidae, subfamily Cicadellinae)

and almost sixty have been identified as *X. fastidiosa* vectors, very few sharpshooter species are present in Europe. Actually, out of nine species of this subfamily recorded in the Fauna Europaea database only one species, *Cicadella viridis*, is widespread and common, though mostly restricted to hygrophilous environments. On the contrary, thirty six spittlebug species (families Aphrophoridae and Cercopidae), are present in Europe and some of them are very common and widespread. Among these, the “meadow spittlebug” *Philaenus spumarius*, already identified as a vector of the CoDiRO strain in Salento, is very common and abundant in diverse ecosystems, and feeds on mono- and dicotyledonous grasses, trees and shrubs. Among the European xylem-sap feeding insects, cicadas (families Cicadidae and Tibicinidae) are represented by tens of species, often with high population level, in the Mediterranean area. Some species, like *Cicada orni* can also be very abundant on olive trees. It can be suggested that, while in Northern and Southern America sharpshooter vectors have primarily been associated with *X. fastidiosa* epidemics, in Europe xylem-sap feeders other than sharpshooters might play a more important role in the spread of this bacterium.

**PHYTOSANITARY REGULATION AGAINST XYLELLA FASTIDIOSA FROM A EUROPEAN UNION PERSPECTIVE.**

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*Xylella fastidiosa* (*Xf*) is a bacterial plant pathogen known to be the causal agent of serious diseases in several relevant crop plants, which are difficult to control and have a high economic impact. Therefore *Xf* is listed in the EU plant health Directive (Council Directive 2000/29/EC) as a quarantine pest not known to occur in the EU, whose introduction into, and spread within, all EU Member States is banned. Moreover, Member States are requested to immediately notify the presence in their territory of *Xf* and they shall take all necessary measures to eradicate this harmful organism. Upon the notification of an outbreak of *Xf* in the region Apulia by the Italian authorities on 21 October 2013, which represents the first confirmed presence of this pest in the Union, the European Commission rapidly adopted provisional emergency measures to prevent the spread within the Union of *Xf* (Implementing Decision 2014/87/EU of 13 February 2014). These measures put restrictions for the movement of plants out of the province Lecce, which could be a pathway for the spread of the bacterium to other areas, and introduced an obligation for Member States to conduct annual surveys for the presence of this bacterium in their territory. Once more information on the strain of *Xf* found in Apulia became available, the Commission adopted more detailed emergency measures (Implementing Decision 2014/497/EU of 23 July 2014) which provide conditions on the import and movement of particular plants which host, or are likely to host this bacterium, its timely identification in the affected areas, as well as its control. The measures include obligations to notify any outbreak, official annual surveys, demarcation of infected areas, sampling, testing and monitoring, and removal and destruction of infected plants. These emergency measures will be updated when more information becomes available, for example with respect to the host range of the bacterial strain identified in Apulia.

**DISEASES INDUCED BY XYLELLA FASTIDIOSA subsp. PAUCA: ECOLOGY, EPIDEMIOLOGY AND MANAGEMENT.**

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The bacterium *Xylella fastidiosa* subsp. *pauca* (*Xf pauca*), restricted to South America (mainly Brazil) up to recently, has also been

reported from Argentina and Paraguay. In Brazil this bacterium causes problem to two economically important crops. i.e. coffee and sweet orange (*Citrus sinensis*). Even though they are genetically close, *Xf pauca* isolates from coffee and citrus do not cause disease in their non-reciprocal hosts. The present work will be focused on the *Xf pauca*-citrus pathosystem based on recent information and scientific work. In 1987, sweet orange plants of commercial orchards located in the Northwest region of the São Paulo state were found to be diseased, showing previously unknown symptoms. Initial hypotheses on the causes of this new disease included mainly abiotic stresses, including nutritional deficiencies. This hypothesis was discarded when epidemiological studies indicated that a contagious and likely vector-borne pathogen was associated with the disease. Tissue grafting from symptomatic plants resulted in transmission of the etiological agent, and electron microscopy showed bacteria colonizing the xylem vessels of infected plants. The fulfillment of Koch's postulates, around 1993, identified the bacterium *X. fastidiosa* as the etiological agent of the disease, which was then named Citrus variegated chlorosis (CVC). Later studies showed that: (i) xylem-sap feeding sharpshooters (Hemiptera:Cicadellidae), now totalizing 13 different species, transmit this bacterium plant-to-plant; (ii) 20% of efficiency of budding transmission even when buds come from asymptomatic but *Xf. pauca*-infected plants are used; (iii) latency period of disease range from ca. six months to years, and seems to be directly correlated with warm temperature and waters stress; (iv) both primary and secondary forms of bacterial transmission by the vectors are important for disease spread in the field. All this information about the CVC pathosystem (a vector-borne disease infecting a graft-propagated perennial plant like citrus) and the significant increase of CVC in São Paulo state at the end of the 1990s, provided scientific and technical support for the mandatory enforcement of production of certified nursery trees (mother citrus plants, rootstock seedlings, bud sticks, and grafted plants) within vector-proof screenhouses since January 2003. In addition to the use of healthy nursery trees the growers implemented the control of vectors, and the voluntary eradication of CVC-diseased trees. This 'technologic package' has helped the growers to continue sweet orange production under CVC pressure. Notwithstanding these efforts, CVC is endemic in all citrus-growing areas of Sao Paulo state and Brazil, but the disease severity varies according to geographic regions. As a consequence, sustainable CVC management has been achieved by researchers and technician. Breeding programs and mass selection in the field under disease pressure has resulted in the identification of CVC-resistant citrus genotypes with economic potential. Basic researches have also produced new molecules with a potential for CVC control.

**SURVEY OF AUCHENORRHYNCHA IN THE SALENTO PENINSULA IN SEARCH OF PUTATIVE VECTORS OF XYLELLA FASTIDIOSA subsp. PAUCA CoDiRO STRAIN. D. Cornara<sup>1</sup>, G. Loconsole<sup>2</sup>, D. Boscia<sup>2</sup>, A. De Stradis<sup>2</sup>, R. K. Yokomi<sup>3</sup>, D. Bosco<sup>4</sup>, F. Porcelli<sup>1</sup>, G. P. Martelli<sup>1</sup> and M. Saponari<sup>2</sup>.**

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*Xylella fastidiosa* (*Xf*) was identified in September 2013 in olive trees affected by the Olive quick decline syndrome (OQDS) in the Salento peninsula (southern Italy) and denoted *Xf* strain CoDiRO.

*Xf* is comprised of a group of genetically diverse bacteria in the class Gammaproteobacteria that causes severe plant diseases in many crops and ornamentals. The bacterium is acquired and transmitted by xylem-sap feeding hemipterans such as sharpshooter leafhoppers (Cicadellidae, Cicadellinae), froghoppers and spittlebugs (Aphrophoridae and Cercopidae) and, possibly, cicadas (Cicadidae and Tibicinidae). Due to the rapid spread and devastation associated with OQDS, a survey of candidate vectors of *Xf* was conducted from September 2013 in the Gallipoli area in accordance to a EFSA list (EFSA, 2013). Four candidate vector species were identified: (i) Aphrophoridae: *Philaenus spumarius* L. and *Neophilaenus campestris* Fallen; (ii) Cercopidae: *Cercopis sanguinolenta* Scopoli; (iii) Cicadidae: *Cicada orni* L. Among these, only *P. spumarius*, the meadow spittlebug, was experimentally proven to be a vector of *X. fastidiosa* strain CoDiRO. A high percentage of meadow spittlebugs collected from OQDS-affected orchards, from May to September 2014, tested positive for *X. fastidiosa* by PCR. Transmission to periwinkle plants was successful. Laboratory tests, so far limited to the *Philaenus*-exposed periwinkle seedlings, will be extended to the entire panel of the host plants (olives, grapes, citrus, oleander and *Prunus* spp.) that were exposed to infectious spittlebugs. Further ongoing experiments include *Xf*-free spittlebugs that were allowed to feed on infected olives and other hosts plants prior to transferring onto receptor host plants. The results so far obtained have shown that olive is a source of inoculum from which *P. spumarius* is able to acquire the bacterium and transfer it to other olives. These data strongly suggest that the main vector of *Xf* in the area of its occurrence is *P. spumarius*. Transmission tests carried out with other xylem sap feeders found in the OQDS area are also discussed.

**OBSERVATIONS ON THE BIOLOGY AND ETHOLOGY OF APHROPHORIDAE: PHILAEUS SPUMARIUS IN THE SALENTO PENINSULA. D. Cornara and F. Porcelli. Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy E-mail: francesco.porcelli@uniba.it**

*Philaenus spumarius*, the meadow spittlebug, was shown to transmit *Xylella fastidiosa* CoDiRO strain; whereas other candidate vectors collected in the OQDS-affected area sporadically harboured *X. fastidiosa*. Therefore, biology and ethology data were collected on the meadow spittlebug in the infected area. Oviposition of *P. spumarius* was observed to occur in autumn-winter, later than reported in the literature. Nymphs were observed in foam nests on weeds and herbs in the olive groves from March to late April. Adults were found from spring to late autumn, although a few adults were always collected during late autumn and winter on weeds and shrubs. Adult presence peaked from late April to July in olives. From late May, *P. spumarius* adults were collected from the canopy of olives and other hosts like lentisk (*Pistacia lentiscus*), myrtle (*Myrtus communis*) and grapevine (*Vitis vinifera*) whereas they were scarce on weeds. Adults moved from olive canopies back to weeds from late July. Mating activities were observed from early May to late September. Observations on feral populations and in leaf cages placed on the branches of field trees showed that the spittlebug aggregated at the shoot tips of olive and the weed *Conyza canadensis*. Data on the biology and host plants of other xylem sap feeders found in the survey are also briefly discussed.

**SOME APPROACHES AIMING AT CITRUS VARIEGATED CHLOROSIS CONTROL IN BRAZIL. A.A. de Souza, M. Cristofani-Yaly, H. Della Coletta-Filho, M. A. Machado. Centro de Citricultura Sylvio Moreira, Instituto Agronomico de Campinas, 13490-000 Cordeópolis, SP, Brazil. E-mail: alessandra@centrodecitricultura.br**

*Xylella fastidiosa* is a phytopathogenic bacterium that causes disease of many different crops worldwide. In Brazil, it is the causal agent of citrus variegated chlorosis (CVC), which is an important disease responsible for economic losses to the citrus industry. Despite the citrus growers are living with this disease in the field, implementing a package management specific for CVC, there is no effective method for its ultimate control in the field. In the Citrus Research Center "Sylvio Moreira" (CCSM, Brazil) we are following two different approaches to avoid disease development in the field and, consequently, decrease the economic losses caused by CVC. One approach consists in citrus breeding. All the cultivars of *Citrus sinensis* (sweet orange), the main citrus species grown in Brazil are susceptible to CVC, whereas other species, e.g. *C. reticulata* and its hybrids, are resistant. Thus, a hybrid citrus population from a cross between sweet orange x tangor (*C. sinensis* x *C. reticulata*) cv. Murcott has been tested in the field and shown to have different levels of CVC resistance and to bear fruits of good quality. The second and new approach consists in the use N-Acetylcysteine (NAC), a cysteine analogue used mainly to treat human diseases, for *X. fastidiosa* and CVC control. We verified that significant symptoms remission and reduced bacterial replication rate were observed when NAC was applied to greenhouse-grown symptomatic plants. Using NAC absorbed to a slow-release fertilizer the lag for symptom resurgence on the leaves after the interruption of the treatment was extended to ca. eight months. These results demonstrated that NAC-fertilizer probably increased the time of NAC availability to the plant, hence it decreased the damage of CVC disease. Thus, NAC-fertilizer or any other compound that would allow a slow release of NAC might represent a real strategy to be applied in the field for controlling CVC. NAC-fertilizer is already been tested in the field on plants showing severe CVC symptoms. Therefore, we expect that the use of these approaches might be a sustainable strategy for controlling CVC, since the current management methods include pruning, use of insecticide and eradication of severely symptomatic plants, which increase the cost of production and cause damage to the environment.

Financial support: FAPESP, CNPq.

**AN INNOVATIVE MONITORING MODEL FOR XYLELLA FASTIDIOSA IN APULIA.** A.M. D'Onghia<sup>1</sup>, F. Santoro<sup>1</sup>, T. Yaseen<sup>1</sup>, K. Djelouah<sup>1</sup>, A. Guarino<sup>2</sup>, A. Percoco<sup>2</sup>, T. Caroppo<sup>3</sup> and F. Valentini<sup>1</sup>. <sup>1</sup>CIHEAM, Istituto Agronomico Mediterraneo di Bari. Via Ceglie 23, 70010, Valenzano (Bari), Italy. <sup>2</sup>Osservatorio Fitosanitario della Regione Puglia, Lungomare Nazario Sauro 45/47, 70121 Bari, Italy. <sup>3</sup>Innovapuglia S.p.A. Strada. Provinciale per Casamassima km. 3,000, 70010 Valenzano (Bari), Italy. E-mail: donghia@iamb.it

*Xylella fastidiosa*, one of the most detrimental bacteria affecting a large number of hosts in the world, has recently been introduced in the EU and the Mediterranean basin, where is associated with the Olive quick decline syndrome (OQDS) a severe disease affecting *Olea europaea* trees. Sound and sustainable surveillance and management of *X. fastidiosa* are based on the timeliness of interventions, on the thorough knowledge of the territory and the evolution of infection over time and space since its outbreak. A monitoring model has been developed for the rapid identification of trees suspected to be infected with no need to move plant material to the laboratory for analysis. This model integrates innovative tools of territorial analyses through photo-interpretation of aerial images in GIS environment, information technology for field data acquisition by smart devices, and innovative diagnostic methods for *in situ* pathogen detection in plant material (DTBIA) and insects (real-time LAMP). *X. fastidiosa* detection in the insects, which are called 'spy insects', represents an effective preventive diagnostic tool that can reveal the presence of the bacterium in pathogen-free areas before symptoms develop on plants. A similar strategy had

previously been developed and successfully applied for monitoring *Citrus tristeza virus* (CTV) in Apulia.

**DRAFT GENOME SEQUENCE OF XYLELLA FASTIDIOSA STRAIN CoDiRO.** A. Giampetruzzi<sup>1</sup>, M. Chiumenti<sup>1</sup>, M. Saponari<sup>1</sup>, G. Donvito<sup>2</sup>, A. Italiano<sup>2</sup>, G. Loconsole<sup>1</sup>, C. Cariddi<sup>3</sup>, G.P. Martelli<sup>3</sup> and P. Saldarelli<sup>1</sup>. <sup>1</sup>Istituto per la Protezione Sostenibile delle Piante del CNR (former Istituto di Virologia Vegetale), UOS Bari, Via Amendola 122/D, 70126 Bari, Italy. <sup>2</sup>Istituto Nazionale di Fisica Nucleare, Via Orabona, 4, 70125 Bari, Italy. <sup>3</sup>Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, Via Amendola 165/A, 70126, Bari, Italy. E-mail: p.saldarelli@ba.iov.cnr.it

Seven complete genomes of *X. fastidiosa* have been determined, including the Citrus variegated chlorosis strain 9a5c, the Pierce's disease strains Temecula 1 and GB514, the almond leaf scorch strains M12 and M23, the oleander strain Ann1, and the mulberry strain MUL0034. Draft genomes of strains from almond, elderberry, mulberry, oak, coffee, pear and sycamore are also available ([www.ncbi.nlm.nih.gov/genome/genomes/173](http://www.ncbi.nlm.nih.gov/genome/genomes/173)). A novel strain (CoDiRO) was isolated from olive trees affected by the quick decline syndrome (OQDS) in southern Italy whose genome organization was investigated by a next generation sequencing (NGS) approach. Three libraries, using DNA extracted from xylem tissues of *X. fastidiosa*-infected and healthy olive plants and from axenic cultures of the CoDiRO strain isolated from periwinkle, were paired-end sequenced by Illumina technology. Libraries from the culture, infected and healthy olive plants contained 9,008,814, 29,096,610 and 28,333,924 reads, respectively. *De novo* assembling of reads from the purified bacterial DNA (library 49I) by SOAPdenovo and Velvet, generated 480 *Xylella*-homologous contigs, having the longest and the best N50 contig size of 255,649 and 176,495 and 73,268 and 76,859 bp, respectively. With both collections the majority (>52%) of the contigs, had a primary hit with members of *X. fastidiosa* subsp. *pauca*, a finding that was confirmed by MLST analysis of seven loci. Integrating contigs from both assembly methods by the CISA package and combining the output with the NGS paired-end reads data by the SSPACE software, generated a set of 45 scaffolds with sizes ranging from 126 to 322,823 bp and an average scaffold size of 54,694 bp. Scaffolds were ordered on the backbone of the *X. fastidiosa* subsp. *pauca* 9a5c genome using MAUVE 2.3.1 software and led to the reconstruction of a preliminary draft genome consisting of a total of 2,462,158 bp (average coverage 252X) with a GC contents of 51.8%. Partial annotation performed by PROKKA, allowed the identification of 6 rRNA genes, 47 tRNA loci, 1 tmRNA and 2,343 coding sequences. Moreover, a single circular contig of 35kb, shares 98% of similarity with the large conjugative 38kb plasmid pXF-RIV, which was predicted to be present in uncharacterized strains of the bacterium, but differs from it for annotated genes of toxin-antitoxin system.

**CONTROL STRATEGIES FOR XYLELLA FASTIDIOSA.** D.L. Hopkins. Mid-Florida Research and Education Center, University of Florida, Apopka, Florida 32703, USA. E-mail: dbop@ufl.edu

*Xylella fastidiosa* causes economic losses in many agriculturally important plants, including almond, blueberry, citrus, coffee, grape, oleander, peach, plum, and several different shade tree species. It is spreading into new hosts and areas, such as olive in Europe. Once *X. fastidiosa* is established in an area, it is very difficult to control. Thus, extensive quarantine efforts have concentrated on prevention of its introduction into new areas. The wide host ranges of both *X. fastidiosa* and its vectors, along with the global movement

of plant material, have made exclusion of the pathogen difficult to maintain. Cold winter temperatures limit the range of the diseases, thus eliminating them in some colder areas. There are several controls that may reduce the losses from diseases caused by *X. fastidiosa*. Systemic insecticides can be used to reduce the overall vector populations and reduce pathogen spread. Other hosts of *X. fastidiosa* should be removed from around the field and infected plants in the field should be rogued regularly. Other stresses on the host plant, such as drought, weeds, and other diseases, should be reduced. Root and stem canker diseases are often found in association with *X. fastidiosa*. Plant resistance is the best solution for diseases caused by *X. fastidiosa*; however, resistance has not been identified for many of the diseases. For example, all sweet orange cultivars are susceptible to citrus variegated chlorosis (*X. fastidiosa* subsp. *pauca*). In grapevine, *Vitis vinifera* cultivars are susceptible to Pierce's disease and resistance is found in other species, which are less favorable for wine production. Control of *X. fastidiosa* in the future could result from genetic engineering used to transfer very specific resistance genes into plants. Several types of genes are currently being tested in grapevine in California for Pierce's disease control. In Florida tests, precision breeding is used to transfer specific resistance genes from grape into susceptible *V. vinifera* grapes. Biological control of diseases caused by *X. fastidiosa* with a benign strain of *X. fastidiosa*, EB92-1\*, is a promising option. EB92-1, a naturally occurring strain of *X. fastidiosa*, may provide effective, environmentally-friendly control of diseases caused by *X. fastidiosa*. EB92-1 colonizes host plants, at a 10-100 fold lower population than pathogenic strains. Biocontrol is probably achieved by some type of cross protection rather than competition with the pathogen. Treating young plants in the greenhouse prior to transplanting into the field is the preferred treatment; however, protection of mature plants already in production is also possible. EB92-1 has provided control of blueberry leaf scorch and Pierce's disease in greenhouse tests. In field tests, EB92-1 has provided control of Pierce's disease in various *V. vinifera* cultivars. EB92-1 can also colonize almond, citrus, olive, plum, and various shade trees, which indicates a possibility for biocontrol in these *X. fastidiosa* hosts.

\* University of Florida (USA) patented strain; Luxembourg Industries, Ltd (Israel) licensee and developer

**IDENTIFICATION AND CHARACTERIZATION OF XYLELLA FASTIDIOSA ISOLATED FROM COFFEE PLANTS IN FRANCE.** B. Legendre<sup>1</sup>, S. Mississippi<sup>1,2</sup>, V. Olivier<sup>1</sup>, E. Morel<sup>2</sup>, D. Crouzillat<sup>2</sup>, K. Durand<sup>2</sup>, P. Portier<sup>3</sup>, F. Poliakov<sup>1</sup>, and M. A. Jacques<sup>3</sup>. <sup>1</sup>Anses, Plant Health Laboratory, 7 rue Jean Dixmèras, 49044 Angers, France. <sup>2</sup>Nestlé Research and Development Center, 101 Av. Gustave Eiffel, Notre Dame d'Oé, 37097 Tours, France. <sup>3</sup>INRA, UMR1345, IRHS, 42 rue Georges Morel, 49071 Beaucauzé, France. E-mail: marie-agnes.jacques@angers.inra.fr

*Xylella fastidiosa* (*Xf*) is a xylem-limited bacterium present in the Americas, that has recently emerged in Taiwan, Iran and Italy. Among the 200 plant species that are susceptible to *Xf* some fruit crops (*Vitis vinifera*, *Prunus persica*, *Citrus sinensis*, *Olea europaea*, *Coffea* spp.), ornamentals (*Nerium oleander*, *Platanus occidentalis*) and forest trees (*Quercus* spp., *Ulmus* spp.) have a major economic importance. *Xf* is a quarantine bacterium for the European Union (EU) and, as such, is listed in the directive 2000/29/EC. A risk of introduction is still pending due to the high number of host plants imported from contaminated areas and to latent infection in symptomless hosts. In 2012 four *Xylella*-infected coffee plants (*Coffea arabica* and *C. canephora*) growing in a confined glasshouse were detected in France. This outbreak was eradicated (EPP0 RS N° 8 2012/165). The objectives of the present study were to isolate the pathogen, confirm its identification, decipher its phylogenetic relationships with other coffee-infecting strains of *Xf*, and gain insight

into host adaptation following genome sequencing. Three *Xf* strains were isolated from coffee plants confirming the previous diagnostic based on immunofluorescence. The strains were characterized by multiplex PCR and by multilocus sequence typing (MLST) based on seven housekeeping genes. Two strains isolated from *C. arabica* imported from Ecuador were allocated to a new genetic lineage closely related to *X. fastidiosa* subsp. *pauca* and the third strain, which was isolated from *C. canephora* imported from Mexico was identified as *X. fastidiosa* subsp. *fastidiosa*. Analysis of the genomic sequences of the two distant strains with publicly available genome sequences confirmed these phylogenetic positions. Analyses of genome sequences in link with host specificity and the development of potential targets for diagnosis will be presented. This study confirms the global diversity of *Xf* and highlights the diversity of the strains isolated from coffee.

**INTERLABORATORY VALIDATION OF MOLECULAR AND SEROLOGICAL DIAGNOSIS OF XYLELLA FASTIDIOSA STRAIN CoDiRO IN SUSCEPTIBLE HOST PLANTS.** G. Loconsole<sup>1\*</sup>, O. Potere<sup>2\*</sup>, T. Elbeaino<sup>3</sup>, D. Frasher<sup>3</sup>, S. Frisullo<sup>4</sup>, F. Palmisano<sup>5</sup>, D. Boscia<sup>1</sup> and M. Saponari<sup>1</sup>. <sup>1</sup>CNR Istituto di Protezione Sostenibile delle Piante del CRN (former Istituto di Virologia Vegetale) UOS Bari, Via Amendola 122/D, 70126 Bari, Italy. <sup>2</sup>Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy. <sup>3</sup>Istituto Agronomico Mediterraneo, Via Ceglie 9, 70100 Valenzano (BA), Italy. <sup>4</sup>Dipartimento di Scienze Agrarie, degli Alimenti e dell'Ambiente, Università degli Studi, Via Napoli 25, 71100 Foggia, Italy. <sup>5</sup>Centro di Ricerca, Sperimentazione e Formazione in Agricoltura, Via Cisternino 281, 70100 Locorotondo (Bari), Italy. E-mail: g.loconsole@ba.iva.cnr.it. \* These authors contributed equally to the work

Accurate detection of harmful plant pathogens that cause severe crop losses is critical for the successful control and management of emerging diseases. Following the recent outbreak of *Xylella fastidiosa* in the Apulia region (southern Italy), diagnostic protocols based on ELISA and conventional PCR were successfully used and adopted for large-scale surveys. However, a validation of these protocols for pathogen detection by different laboratories in diverse susceptible hosts is periodically needed to guarantee optimum sensitivity and reliability. Thus, molecular and serological protocols for the detection of *X. fastidiosa* strain CoDiRO in plant tissues were compared in four different laboratories for a reliable estimation of the performance of each method. A panel of blind samples from healthy and CoDiRO-infected hosts was tested. These hosts included naturally infected olive, oleander, cherry, almond, *Polygala myrtifolia* and *Acacia saligna*, while leaf extracts from grapevine and citrus, which are apparently not susceptible to infection, were artificially spiked with DNA from a standard aliquot of heat-inactivated bacterial cell suspension. Assays included in the ringtest were conventional PCR using two primer sets, quantitative (q) PCR using previously developed molecular markers, and ELISA using a commercial kit (Loewe Biochemica GmbH, Germany). The sensitivity of the test was determined using 10-fold serial dilutions of an inactivated suspension of CoDiRO strain cells of known concentration, designed to determine the detection limits of the different methods. Results showed that *X. fastidiosa* was correctly identified by ELISA and PCR in all plant matrices, including the citrus and grape extracts spiked with the bacterial suspension. None of the samples known to be *X. fastidiosa*-free gave false positive reactions. CTAB-based procedure proved most suitable for the isolation of high quality DNA templates from all plant matrices based on amplification of a plant internal positive DNA control targeting the cytochrome oxidase gene. As to sensitivity, ELISA and conventional PCR showed a similar level of detection limit with clear positive reactions up to a dilution of 10<sup>-5</sup>, while qPCR was 10<sup>2</sup> times more



sensitive than either method. This inter-laboratory validation has provided standard guidelines which are brought to the attention of the European Union for their use in *X. fastidiosa* monitoring programs to be implemented in the Mediterranean basin following the Apulian outbreak.

**THE OLIVE QUICK DECLINE SYNDROME: STATE-OF-THE-ART.** G.P. Martelli. *Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro. Via Amendola 165/A, 70126 Bari, Italy. E-mail: giovanni.martelli@uniba.it*

The unexpected and unwelcome arrival of *Xylella fastidiosa* (*Xf*) in the Salento peninsula, the heel of the boot of Italy, has created an unprecedented turmoil because of: (i) the dramatic damage suffered by olive groves where the bacterium has established itself; (ii) the alarm that this record has created both in a country (Italy) where the olive/oil industry is a primary asset, and in the European Union, which is facing the first confirmed record in its territory of this alien and much feared pathogen. To the observer, the disease looked impressively severe and destructive especially when it affected aged (centenarian) and large trees. At a close examination, many of such trees appeared to suffer because of the concomitant presence of three quite different agents: (i) the leopard moth (*Zeuzera pyrina*), a lepidopteron endemic in the area, whose larvae drill galleries in the branches and trunks of olives; (ii) a set of xylem-inhabiting fungi of different genera (*Phaeoacremonium* and *Phaemoniella*, in particular) which invade the sapwood and take advantage of the moth galleries to invade the wood; (iii) *Xf* which, like the mentioned fungi, inhabits the xylem vessels. Since these findings suggested that the disorder was consequential to a complex of causes, it was called “Olive quick decline complex” or, in Italian, “Complesso del disseccamento rapido dell’olivo”, the abbreviation of which, CoDiRO, has been adopted to designate the *Xf* strain associated with it. As time went by and a better insight into the disease was gained with field and laboratory observations, it became evident that the role of the leopard moth was minor, as shown by the fact that no galleries are present in younger olive groves which, nonetheless, are diseased, whereas the fungi could play the role of aggravators. It ensues that, currently, the disease is referred to as “Olive quick decline syndrome” (OQDS). We strongly suspect that *Xf* is by itself capable of crippling the invaded olives. Experimental evidence of this is being sought and will be secured with the pathogenicity tests that have been initiated by prick inoculating (successfully) olive rooted cuttings with bacterial suspensions from pure colonies. *X. fastidiosa* was stepped upon quite by chance when the symptomatology characterizing OQDS and the quick rate at which it appeared to be spreading suggested to look for its presence taking advantage of the availability of an old ELISA kit that had been used years before for the identification of *Xf* in scorched almond leaves from Turkey. The serological test was, much to our alarm, positive. When the identification was confirmed by PCR we knew that we were in trouble, but we also knew that it was essential to move fast and gather as much information as possible on the distribution of the bacterium, the nature (taxonomic allocation) of its strain, the alternative hosts and the vectors. All of this counting on a limited bacteriological experience (we, the early nucleus of researchers, are virologists) and on even more limited financial resources. Most of these tasks have now been accomplished: (i) efficient and reliable “traditional” detection methods (ELISA and PCR) have been applied and validated with an interlaboratory ring test, while novel diagnostic protocols, i.e. real time loop-mediated isothermal amplification (RT-LAMP) and direct tissue blot immunoassay (DTBIA) have been developed; (ii) the bacterium was isolated in pure culture first from periwinkle plants that had been exposed to *Xf*-carrying spittlebugs (*Philaenus spumarius*), then from

olive, oleander, almond, cherry, myrtle-leaved milkwort (*Polygala myrtifolia*) and coastal rosemary (*Westringia fruticosa*); (iii) multilocus sequence typing ascertained that the CoDiRO strain belongs to the subspecies *pauca*. In fact, the concatenated sequences of the seven genes showed that this strain is a divergent *Xf pauca* variant identical to a strain infecting oleander in Costa Rica (its place of origin?). This taxonomic allocation was confirmed by the draft genome sequence obtained from the DNA extracted from an infected olive tree and a bacterial culture; (iv) extensive surveys for the presence of the bacterium showed that its alternative hosts are shrubs (e.g. oleander, myrtle-leaved milkwort, broom, etc.) and some *Prunus* species (almond, cherry), whereas ornamentals (several Palmaceae, succulent plants and conifers) and a number of monocotyledonous and dicotyledonous weeds (over 100 species in 40 families) are not. Thus, olive itself seems to be the major source of inoculum for secondary spreading; (v) the meadow spittlebug (*P. spumarius*) has a strict association with olive (hundreds of individuals colonize the trees from spring throughout summer) and with *Xf* (the rate of *Xf*-carrying adults is always very high, up to 100%). This frog hopper was able to transmit experimentally the CoDiRO strain from olive to periwinkle and from olive to olive, thus accrediting itself as the major and most efficient vector currently identified. In conclusion, the epidemiological data acquired are sufficient for envisaging an integrated control strategy based on chemical and agronomical measures, in the attempt to restrain OQDS within the borders of the currently infected area.

**IDENTIFICATION AND CHARACTERIZATION OF FUNGAL SPECIES ASSOCIATED WITH THE QUICK DECLINE OF OLIVE.** F. Nigro, I. Antelmi, A. Ippolito. *Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro Via Amendola 165/A, 70126 Bari, Italy. E-mail: franco.nigro@uniba.it*

A severe and rapidly spreading decline of olive trees has occurred in a large area of the Salento peninsula of Apulia (southern Italy). The disease, named Olive Quick Decline Syndrome (OQDS), is characterized by rapid dieback of shoots, twigs and branches, eventually leading to death of the tree. Leaf tips and margins turn dark yellow to brown, tissue discolouration then spreads inward, leading to necrosis of the blade. Symptoms usually progress in severity from the older to the younger leaves on a branch; dried leaves, as well as mummified drupes remain attached to symptomatic shoots and branches. Leaf symptoms may be localized to a single limb or sector of the tree, or may extend to the whole canopy. Trunks, branches and twigs viewed in cross section show discolorations of a few or most of the vascular elements. Sapwood and vascular cambium show intense dark streaking and/or light brown tissue discoloration. Numerous galleries of the leopard moth, *Zeuzera pyrina*, and bark beetles occur on the trunks, branches and twigs of affected plants. The quarantine pathogen *Xylella fastidiosa* and several fungal species were found associated with the OQDS. The objectives of this work were: (i) to determine the kind and distribution of the fungal species associated with OQDS; (ii) to assess the phenotypic and genotypic diversity of the species inside and outside the areas infected by *X. fastidiosa*. The presence of *X. fastidiosa* in the samples was ascertained by PCR and fungal isolations from discolored sapwood and bark were made on different agarized media. *Phaeoacremonium parasiticum*, *P. aleophilum*, *P. rubrigenum*, *P. alvesi* and *Pleruostomophora richardsiae* were isolated mainly from the xylem and identified based on morphometric characters and sequencing of ITS,  $\beta$ -tubulin, and TEF genes. These fungi were also isolated from declining olive trees outside the infected area, although less frequently. Molecular and morphometric data, also confirmed the occurrence of *Neofusicoccum mediterraneum*, *N. australe*, and *N. vitifusiforme*, both in discolored sapwood and in

cantered bark. Isolation of *Neofusicoccum* spp. from sapwood of declining trees outside the *Xylella*-infected areas was sporadic. Fungal isolates morphologically similar to *Phaeoconiella* spp. were recovered only from sapwood of young and aged trees positive for *X. fastidiosa* in the infected areas. Sequence analysis of their 26S, LSU, ITS1, ITS2 and 5.8S genes, disclosed a low similarity level with the sequences available in databases. Notwithstanding the morphological similarity with the yeast-like culture of *Phaeoconiella*, this fungus should be regarded as an undescribed species belonging to the *Coelomyces* group. Pathogenicity test are in progress on *Xylella*-free olive plants, to evaluate the role of *Phaeoacremonium*, *Neofusicoccum*, and the undescribed fungal species in the OQDS.

**EPPO SUPPORTING THE EVOLUTION OF PHYTOSANITARY SYSTEMS IN MEMBER COUNTRIES. F. Petter, F. Grousset, M. Suffert.** *European and Mediterranean Plant Protection Organization. E-mail: petter@epppo.int*

The trade of plants for planting has led to introductions of pests into the EPPO region in recent years. At the EPPO Council Colloquium in 2009, concerns were raised about the efficacy of the current plant health systems in place in the EPPO region to deal with the risks presented by plants for planting. The EPPO Study on the Risk of Imports of Plants for Planting was consequently launched. The main findings and outcomes of this study will be presented. A pathway/commodity approach followed for the identification of potential threats to specific crops will also be presented, and the challenges posed by this approach in the current phytosanitary context will be discussed.

**HISTORICAL PERSPECTIVES ON XYLELLA FASTIDIOSA AND THEIR RELEVANCE FOR THE FUTURE. A.H. Purcell.** *Department of Environmental Science, Policy and Management, University of California, Berkeley, CA 94720, USA. E-mail: abpurcell@berkeley.edu*

*Xylella fastidiosa* (Xf) is a bacterium that continues to expand the range of plant hosts, colonizes and causes severe “new” diseases in some of its new host associations. In addition, some long-recognized diseases caused by Xf have expanded their geographic range. One of the most dramatic recent examples of a new plant association is with olive (*Olea europaea*) in southern Italy. The history since the 1880s of research on diseases caused by Xf demonstrates the importance of the persistence and originality of Xf research to prevent or manage these diseases. Specific examples illustrate the impacts of five research topics: (i) disease recognition and description; (ii) proof of the cause of disease; (iii) identification of how Xf and its diseases spread (epidemiology or ecology); (iv) identifying what reduces disease spread and (v) identifying what eliminates or reduces Xf from plants. When N.B. Pierce characterized the “California vine disease” after the first documented epidemic of this disease eliminated commercial viticulture from the Los Angeles basin of southern California, he only speculated that the pathogen was a microbe. However, Pierce’s careful descriptions and distinctions from symptomatically similar grape diseases like esca enabled further research on what W.B. Hewitt later named as Pierce’s disease (PD). From the late 1930s until the mid-1970s, a virus was assumed to cause PD. Spatial patterns of PD in vineyards were clues that led to identifying xylem sap-feeding specialists such as sharpshooter leafhoppers (Cicadellinae) and spittlebugs (Cercopidae) as vectors of the PD “virus”, which also caused alfalfa dwarf disease (AD). The spatial patterns of PD in vineyards near certain habitats that supported vector leafhoppers vines explained why removing PD-diseased vines did not reduce disease spread. In Brazil during the 1990s, analyses of spatial

patterns of citrus variegated chlorosis (CVC) suggested that tree-to-tree spread of the pathogen (then proven to be Xf) caused most spread of CVC, even though Xf was shown to be able to infect a wide range of weed species. Production of Xf-free nursery plants and the careful annual removal of diseased plants or branches with very early symptoms in older (>3 years old) trees also could reduce CVC spread. Spatial patterns of PD in the United States suggested and later experiments concluded that winter cold severity limited the occurrence of PD, but that winter climate is not limiting for Florida. Research in the early 1970s consistently associated bacteria with PD, and the cultivation of Xf in the late 1970s enabled proof that Xf caused PD and triggered the development of sensitive serological and molecular detection methods and tools to differentiate strains of the bacterium. Culturing confirmed earlier findings that used vector transmission experiments during the 1940s that many symptomless plant species supported multiplication of the PD “virus” to various degrees but did not support the indefinite colonization of most symptomless hosts. The first complete genome sequence of a CVC strain in Brazil in 2000 opened new molecular approaches to learn more about the physiology of Xf. Molecular methods pioneered the discoveries that identified multiple promising new approaches to control Xf-caused diseases. The epidemics of CVC in Brazil (1990s) and PD in southern California (~1998 to 2008) spread by a newly invasive vector species (*Homalodisca vitripennis*) in California, massively increased funding for research on all aspects of possible ways to control Xf in Brazil and the United States. These include new grape varieties resistant to Pierce’s disease and quarantines and insecticidal control for *H. vitripennis*. I offer several conclusions and unresolved issues to consider for future research, based on the history of research accomplishments against Xf: (i) proof of the role of Xf in a new disease is a priority because it is essential to the entire research effort; (ii) the ecological components of a Xf-induced disease such as PD vary among different regions. Differences in climates, vectors, outside and within-crop habitats are just a few examples of drastic changes in the basic ecology of PD from one region to another, so fresh research and new ideas will be required for each new outbreak. Systematic data collection to describe disease progress in space and time is essential for understanding how Xf-diseases spread (epidemiology); (iii) the establishment of Xf in Europe should have been expected for many decades. How this occurred in southern Italy is still unknown. The tools and knowledge to quickly confirm the suddenly widespread establishment of Xf in Apulia and the detection of PD and almond leaf scorch (ALS) in Iran and ALS in Turkey depended on sustained research on Xf in Brazil and the USA. Scientific knowledge of Xf was helpful but not adequate by itself to control the newly emerged CVC disease in Brazil, which applied massive new research efforts and coordinated management schemes to control CVC. The same will be true for Europe and elsewhere, and certainly not just for olive and a few other economically important crop plants; (iv) we can expect “new” diseases caused by Xf will continue to emerge because of new encounters of novel strains of Xf with plants that evolved outside the Americas. Some “new” diseases may have been long present but not recognized. This was true for PD in the southeastern USA before 1950 and probably for coffee in Central America currently, if not also for Brazil; (v) we could hugely profit from discovering new ways to kill or block survival of Xf within plants. Chemicals, including insecticides against vectors of Xf have had little success for control of diseases caused by Xf. The control of *H. vitripennis* populations outside vineyards was an exceptional success for southern California viticulture but has not worked in other regions. More effort and original thinking is clearly needed on chemical control of Xf in plants. How freezing kills Xf in dormant grapevines is unknown. Discovering scientific explanations of freezing therapy might be very useful; (vi) plant breeding is a publically ignored or distrusted crop science but clearly the most important, especially dating back to pre-history. For tree and vine crops, breeding new varieties resistant to Xf will require long and expensive research. It is well worth doing; (vii) some research

projects succeeded with minimal financial support, but the broadest successful discoveries and developments required sustained funding that attracted talented researchers with new - often sophisticated - approaches and fresh ideas. Many projects led nowhere, but the successes overshadowed the failures.

**ISOLATION, GENOTYPE AND PRELIMINARY DATA ON THE PATHOGENICITY OF XYLELLA FASTIDIOSA CoDiRO STRAIN.** M. Saponari<sup>1</sup>, G. Loconsole<sup>1</sup>, R. Almeida<sup>2</sup>, H.D. Coletta-Filho<sup>3</sup>, G.P. Martelli<sup>4</sup> and D. Boscia<sup>1</sup>. <sup>1</sup>Istituto di Protezione Sostenibile delle Piante del CNR, Sezione di Bari Via Amendola 165/A, 70126 Bari, Italy. <sup>2</sup>Department of Environmental Science, Policy, and Management, UC Berkeley, California, USA. <sup>3</sup>Centro de Citricultura Sylvio Moreira IAC, Cordeiropolis, SP, Brazil. <sup>4</sup>Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy. E-mail: m.saponari@ba.ipv.cnr.it

The Olive quick decline syndrome (OQDS) represents the first outbreak of *Xylella fastidiosa* (*Xf*) in Europe. Disease symptoms include extensive leaf scorching and branch dieback, discoloration of the vascular system and a generalized progressive decline that leads the plants to death. To determine the role of *Xf* in the OQDS aetiology, pure cultures of the local bacterial strain (CoDiRO) were obtained and used in greenhouse pathogenicity tests. Axenic cultures were readily established from oleander and periwinkle, whereas initial attempts to isolate *Xf* from infected olives failed due to the heavy contamination by bacteria other than *Xf*. This impairment was overcome by imprinting on BCYE medium the freshly cut surface of twigs from infected olive, almond, cherry, myrtle-leaved polygala (*Polygala myrtifolia*) and coastal rosemary (*Westringia fruticosa*). Pure cultures were obtained from all these hosts. Bacterial DNA was isolated from all colonies, purified, partially sequenced and phylogenetic comparisons were made using isolates from different hosts and infection foci. Molecular data showed that the Apulian *Xf* isolates are genetically related to *Xf* subsp. *pauca* and have a high nucleotide identity to one other, supporting the notion that field infections are elicited by the same strain. Bacterial suspensions from cultures recovered from olive were used for pathogenicity tests with inoculation in triplicate of the following hosts: olive (cv. Leccino) and periwinkle seedlings, self-rooted cuttings of grapevine (cv. Cabernet), and *in vitro*-propagated plantlets of GF677 (*Prunus amygdalus* x *P. persica*). All hosts were inoculated by placing a drop of a cell suspension on their stem, below a leaf petiole, followed by pricking with a sterile syringe needle at three inoculation points per plant. Systemic *Xf* infection was recorded in all periwinkle plants one month post inoculation (mpi), and two mpi in the petioles of the olive and GF677 leaves just above the inoculation site. No *Xf* was detected in grapevine petioles. Sampling at 5 and 10 cm above the inoculation site showed that the bacterium had spread from it only in olives. Observations at two mpi showed that systemically infected periwinkles and locally infected olives were still symptomless, whereas GF677 showed deformation and scorching of apical leaves. These results, though preliminary, are encouraging as they experimentally prove the infectivity of the CoDiRO strain. Greenhouse and growth chamber incubation of *Xf*-inoculated plants are continuing with infections routinely monitored by PCR and visual inspections for symptom development. These experiments are in agreement with field observations that olive and *Prunus* are susceptible to CoDiRO strain whereas grapevines are not.

**RISK ASSESSMENT OF XYLELLA FASTIDIOSA AT THE EUROPEAN FOOD SAFETY AUTHORITY.** G. Stancanelli<sup>1</sup>,

R. Almeida<sup>2</sup>, D. Bosco<sup>3</sup>, C. Bragard<sup>4</sup>, D. Caffier<sup>5</sup>, J. C. Gregoire<sup>6</sup>, S. Parnell<sup>7</sup>, G. Strona<sup>8</sup>, O. Mosbach-Schulz<sup>9</sup>, E. Czwienczek<sup>1</sup>, G. Hollo<sup>1</sup>. <sup>1</sup>European Food Safety Authority, ALPHA unit, Plant Health Team, Parma, Italy. <sup>2</sup>Department of Environmental Science, Policy and Management, University of California, Berkeley, USA. <sup>3</sup>Department of Agricultural, Forest and Food Sciences, University of Turin, Turin, Italy. <sup>4</sup>Université Catholique de Louvain, Belgium. <sup>5</sup>High Council for Biotechnology, France. <sup>6</sup>Rotamsted Research, Harpenden, United Kingdom. <sup>7</sup>Institute for Environmental Sustainability, Joint Research Centre of the European Commission, Ispra, Italy. <sup>8</sup>European Food Safety Authority, AMU unit, Parma, Italy. E-mail: alpha@efsa.europa.eu

The role of the European Food Safety Authority (EFSA) is to assess and communicate on risks associated with the food chain, animal and plant health for the European Union (EU) territory. The EFSA Scientific Panel on Plant Health (PLH Panel), composed by 21 independent scientific experts, assesses the risk to plant health and the environment by plant pests and is supported in its tasks by dedicated Working Groups including domain experts and by the EFSA scientific units. Following the discovery of the *Xylella fastidiosa* outbreak in Apulia in October 2013, EFSA has received in November 2013 a request from the European Commission Directorate General Health and Consumers to provide an urgent scientific advice on host plants, entry and spread pathways and risk reduction options for *X. fastidiosa*. This advice was published at the end of November 2013 as an EFSA statement including a preliminary review of host range, vectors, entry and spread pathways and risk reduction options for *X. fastidiosa*. Known hosts of *X. fastidiosa* include many cultivated and spontaneous plants common in Europe, however a range of European wild plant species would meet this bacterium for the first time, increasing uncertainty on the host range. All xylem-fluid feeding insects in Europe should be regarded as potential vectors of *X. fastidiosa*, including insects from the families Cicadellidae, Aphrophoridae, Cercopidae, Cicadidae and Tibicinidae. The main entry pathway for *X. fastidiosa* is the movement of plants for planting. Infective vectors transported on plant consignments are also of concern. The only route for natural spread of *X. fastidiosa* is by insect vectors that generally fly short distances, but can be transported by wind over long distance. The movement of infected plants for planting is the most efficient way for long-distance dispersal of *X. fastidiosa*. Strategies for prevention of introduction from areas where the pathogen is present and for containment of outbreak should focus on the two main pathways and be based on an integrated system approach combining, when applicable, the most effective options. In addition EFSA was also requested to perform a complete pest risk assessment and an evaluation of risk reduction options for *X. fastidiosa*. This work is ongoing and is expected to be completed by November 2014, including a comprehensive literature review on the host plants, vectors and global occurrence of *X. fastidiosa* and its subspecies and the consideration of updated knowledge on the *X. fastidiosa* outbreak in Apulia from the ongoing researches and surveys. In addition, EFSA is also cooperating with Apulian plant health and research institutions as well as with other European research institutes and with the Joint Research Centre of the European Commission in pilot studies on the host susceptibility and spread patterns of *X. fastidiosa* in Apulia.

**MODELLING THE SPREAD OF XYLELLA FASTIDIOSA IN APULIA, ITALY.** S.M. White<sup>1,2</sup>, J.M. Bullock<sup>1</sup>, D.A.P. Hooffman<sup>1</sup>, D.S. Chapman<sup>3</sup>. <sup>1</sup>Centre for Ecology and Hydrology, Benson Lane, Wallingford, Oxfordshire, OX10 8BB, UK. <sup>2</sup>Mathematical Institute, University of Oxford, Andrew Wiles Building, Radcliffe Observatory Quarter, Woodstock Road, Oxford, Oxfordshire, OX2 6GG, UK. <sup>3</sup>Centre for Ecology and Hydrology, Bush Estate, Penicuik, Midlothian, EH26 0QB, UK. E-mail: steven.white@ceb.ac.uk

*Xylella fastidiosa* (*Xf*) is a xylem-limited Gram-negative bacterium and the recognized agent of a number of severe diseases, among which Pierce's disease of the grapevine, leaf scorch of almond, oleander and coffee, citrus variegated chlorosis, and other disorders of perennial crops and landscape plants. Once restricted to the Americas, the bacterium was discovered near Lecce (Apulia, southern Italy) in 2013, and since the initial outbreak, it has spread and affected 8,000 hectares of olive trees in Apulia. *Xf* is transmitted by various species of sap-sucking hopper insects. Infection occurs after a vector has fed on an infected plant and then subsequently feeds on a healthy plant. *Xf* has a very broad range of known host plants, including many grown for agricultural production, and hence the disease could have a large impact on food production. Importantly,

the sap-sucking hopper insects found in the EU that could potentially carry the disease are likely to have different feeding habits and patterns, thus making spread predictions difficult. These facts suggest that the potential spread of *Xf* is of great concern. In this talk we will present a model for the spread of *Xf* throughout the Apulia region. By first considering a simplification of an established multi spatial scale model, we parameterise the infection dynamics using field data. These dynamics are then coupled with a dispersal kernel, and the current distribution and intensity of host plant species throughout the region, to realistically represent the potential spread. We present our case scenario results as well as the impacts of roguing (infected plant removal), which have significant effects on the spread.

## POSTERS



**1. GENOME-WIDE TRANSCRIPTOME ANALYSIS OF OLIVE LEAVES AFFECTED BY QUICK DECLINE SYNDROME.**  
**I. Antelmi, R.M. De Miccolis Angelini, S. Pollastro, F. Nigro.** *Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy. E-mail: franco.nigro@uniba.it*

A severe disease of olive trees has recently occurred in Apulia, in whose aetiology a strain (CoDiRO) of *Xylella fastidiosa* is implicated in association with fungal species of the genera *Phaeoacremonium* and *Neofusicoccum*, or still undescribed. The disease, named Olive quick decline syndrome (OQDS), is characterized by a rapid die-back of shoots, twigs and branches, eventually leading to death of the tree. Sapwood and vascular cambium of affected trees show intense dark streaking and/or light brown tissue discoloration. A deep sequencing transcriptome analysis was carried out by comparing the expression profile of four sets of leaves collected from olive trees of cv. Ogliarola di Lecce: (i) healthy (C1); (ii) infected by *Xylella* without discolored sapwood (C4); (iii) healthy (C2); (iv) infected by *Xylella* with discolored sapwood (C3). The presence/absence of *X. fastidiosa* in the samples was ascertained by PCR. Total RNA was extracted from the midrib of the leaves and the corresponding expression libraries were constructed. The high-throughput sequencing of the libraries (RNA-seq) was performed on a HiScanSQ Illumina platform. Data were analyzed by using specific commercial software for RNA-Seq analyses. Transcripts showing differential expression between samples were annotated according to the three main vocabularies of gene ontology (GO): cellular component, biological process and molecular function. BlastX analysis, GO terms mapping and annotation analysis were done using the Blast2GO software. Moreover, GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were performed. A total of 85 million single-end 51 bp reads of high quality score (Q40 = 1 error/10<sup>4</sup> bases) were mapped on the reference transcriptome of olive (*Olea europaea* subsp. *europaea*). Normalized gene expression values (RPKM) were assessed for each of the 81,020 predicted transcripts of olive in the four libraries analyzed and compared. Comparing the samples, 700, 780, 995, and 951 hypothetical transcripts differentially expressed (DE) were found for the comparison C1:C4, C2:C3, C4:C3, and C1:C2, respectively. A high proportion of annotated up-regulated genes referred to binding, catalytic activity, pyrophosphatase and hydrolase activity, transporter activity, and oxidoreductase activity. *De novo* assembly was made for a total of 28.5 million un-mapped reads. Preliminary data show the presence of transcripts related to fungal species involved in the browning of the xylem vessels. Transcripts of uncultured bacteria were also found.

**2. XYLELLA FASTIDIOSA NOT FOUND ON OLIVE AND OLEANDER IN A CROATIAN 2014 OFFICIAL SURVEY.**  
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Following the Commission Implementing Decision 2014/87/EU, an official survey for the presence of *Xylella fastidiosa* in plants and plant products has been undertaken since February 2014 in six counties along the coastal areas of Croatia, as a preventive measure against the introduction of this harmful organism. Since the main entry pathway for the specified organism is the movement of plants for planting, the survey was focused on young plants of *Nerium oleander* and *Olea europaea*, based on the data on imports of plant material from the past few years. These plants are present in all

urban and semi urban zones used for horticultural landscaping, as well as along roads and highways. The survey was also extended to the intensive and traditional olive plantations of Dalmatia and Istria. Visual inspections and sampling were done by the Institute for Plant Protection (PPI) and the Regional Phytosanitary Inspection Units. Survey covered approximately 40 towns and cities that are tourist-oriented and characterized by high traffic and patchy horticultural decoration with some of the main host plants of the targeted harmful organism. The main and other potential host plants were visually inspected for symptoms of leaf scorching, dying or decline. Samples displaying these symptoms were collected for serological and molecular assays. A total of 80 samples of oleander and olive were analysed. DAS-ELISA was used as the first method of detection whereas PCR with specific primers amplifying the 16Sr RNA and *gyrB* genes was used for confirmation. The extensive visual inspections and laboratory assays carried out in 2014 showed that *X. fastidiosa* is not present in olive and oleander in Croatia.

**3. THE POSSIBLE ROLE OF OLEANDER IN THE EPIDEMIOLOGY OF XYLELLA FASTIDIOSA IN THE SALENTO PENINSULA.** **D. Boscia<sup>1</sup>, O. Potere<sup>2</sup>, G. Loconsole<sup>1</sup>, M. Sapronari<sup>1</sup>, A. Delle Donne<sup>3</sup>, L. Susca<sup>2</sup>, G. P. Martelli<sup>2</sup>.** *<sup>1</sup>Istituto di Protezione Sostenibile delle Piante del CNR, Sezione Virologia Bari, Via Amendola 165/A, 70126 Bari, Italy. <sup>2</sup>Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy. <sup>3</sup>Regione Puglia, Area Politiche per lo sviluppo rurale, Ufficio Provinciale Agricoltura di Lecce, viale Aldo Moro, 73100 Lecce, Italy. E-mail: d.boscia@ba.ivv.cnr.it*

Oleander (*Nerium oleander*) is a host of different genotypes of *Xylella fastidiosa* (*Xf*) including the CoDiRO strain, which is associated with the quick decline syndrome of olive (OQDS) in the Salento peninsula, and was shown by MLST analysis to belong to the subspecies *pauca*. The CoDiRO strain, however, is molecularly distinct from the *Xf pauca* isolate reported from coffee and citrus, of which oleander is not a host. Interestingly, a molecular variant of *Xf pauca* identical to CoDiRO has been found in oleander in Costa Rica (Nunney L. and B. Ortiz, personal communication). Oleanders are widely grown in Apulia, to whose climate they are perfectly adapted, where they are used as ornamental plants in public and private gardens, in city streets and as edges in roads and highways. The frequent presence of oleanders in areas affected by OQDS prompted an investigation for ascertaining their role, if any, in the epidemiology of the CoDiRO strain. A preliminary survey was therefore carried out based on random sampling and visual inspections in: (i) an area where OQDS is well established (records dating back to the past three years), (ii) areas of recent disease expansion. Leaf symptoms of oleander consistently associated with *Xf* infections are apical leaf scorch and necrosis running lengthwise along the leaf margin. Apical scorching can be also induced by other causes (e.g. hot dry winds, salty winds, nutrient deficiency/toxicity), while marginal necrosis seems to be associated exclusively with *Xf* infections, thus can be taken as a *Xf*-specific symptom. Moreover, affected plants show also dieback of the whole canopy and often react by pushing suckers, thus reminding the behaviour of OQDS. Results of laboratory analysis (ELISA and real-time PCR) disclosed a significant incidence of *Xf* infection, almost always symptomatic, only in the area where OQDS incidence is close to 100% and the presence of *Xf* dates back to three years or more. By contrast, in newly infected areas (less than a year) *Xf* was still absent in oleander, whereas other alternative hosts, e.g. *Polygala myrtifolia*, were widely infected. One possible explanation for this delayed infection of oleander can be found in the behaviour of the main vector of the CoDiRO strain, the spittlebug *Philaenus spumarius* which, based on observations made in the course of experimental transmission trials, seem to dislike oleander. Taken as a whole, these observations

seem to support the notion that oleander may have a minor, if not negligible role in the epidemiology of *Xf* in the Salento peninsula.

**4. FIELD OBSERVATIONS ON THE BEHAVIOUR OF DIFFERENT OLIVE CULTIVARS IN RESPONSE TO *XYLELLA FASTIDIOSA* INFECTIONS.** D. Boscia<sup>1</sup>, M. Saponari<sup>1</sup>, F. Palmisano<sup>2</sup>, G. Loconsole<sup>1</sup>, G.P. Martelli<sup>3</sup> and V. Savino<sup>3</sup>. <sup>1</sup>Istituto per la Protezione Sostenibile delle Piante del CNR, Sezione Virologia Bari, Via Amendola 165/A, 70126 Bari, Italy. <sup>2</sup>Centro di Ricerca, Sperimentazione e Formazione in Agricoltura "Basile-Caramia", Via Cisternino, 281, 70010 Locorotondo, Italy. <sup>3</sup>Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy. E-mail: d.boscia@ba.ivv.cnr.it

The Italian olive germplasm comprises one of, if not the highest number of autochthonous cultivars in the world. The recent finding of severe infections of *Xylella fastidiosa* in *Olea europaea*, a relatively new host for this harmful pathogen, prompted a survey for a preliminary evaluation of the susceptibility of different of its cultivars. Whereas for other crops, such as citrus and grapevine, a wide spectrum of resistance to susceptibility to *X. fastidiosa* is found, as yet no such information exists for olive. Surveys were therefore conducted in selected orchards located in the area affected by the Olive quick decline syndrome (OQDS), where the widely grown cvs Ogliarola salentina and Cellina di Nardò coexist with those used for new plantations (25-year-old or younger), i.e. Leccino, Nociara, Carolea, Coratina and Nocellara del Belice. Disease symptoms were scored and used for a rough categorization of cultivar response to OQDS. Under natural infection conditions, ancient trees of cvs Ogliarola salentina and Cellina di Nardò appeared highly susceptible to the bacterium and were severely affected by OQDS regardless of the age of the orchard (century old or of 25 to 30 years of age). By contrast, trees of cv. Leccino, grown in the same conditions, had definitely milder symptoms, since desiccated twigs in the canopy were few and scattered. Although the number of inspected trees of cvs Nociara, Carolea and Nocellara was relatively limited, all these cultivars showed symptoms of intermediate severity, certainly milder than those displayed by cvs Ogliarola salentina and Cellina di Nardò. Quantitative PCR assays for the estimation of the bacterial concentration in the xylem tissues of diseased plants, disclosed that cv. Leccino's trees had a significant lower concentration of the bacterium, with quantitation cycle being of 3-4 points lower than the values obtained with preparations from cvs Ogliarola salentina and Cellina di Nardò. These preliminary observations are reassuring, for they suggest that a differential susceptibility to *X. fastidiosa* exists among olive cultivars, and encourage further investigations, hopefully confirmatory, aimed at determining whether the Salentinian olive industry could benefit from a modification of its current varietal platform.

**5. IDENTIFICATION AND DIVERSITY ASSESSMENT OF *XYLELLA FASTIDIOSA* FROM INFECTED OLIVE TREES IN APULIA (SOUTHERN ITALY).** I. Camele<sup>1</sup>, S.M. Mang<sup>1</sup>, H.S. Elshafie<sup>1</sup>, M. Sasso<sup>1</sup> and S. Frisullo<sup>2</sup>. <sup>1</sup>School of Agricultural, Forestry, Food and Environmental Sciences, University of Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy. <sup>2</sup>Department of Agricultural, Food and Environmental Sciences, University of Foggia, Via Napoli, 25, 71121 Foggia, Italy. E-mail: ippolito.camele@unibas.it.

*Xylella fastidiosa* (*Xf*) is a quarantine xylem-limited Gram-negative bacterium that causes economically important diseases and occurs as four subspecies: *fastidiosa*, *multiplex*, *pauca* and *sandyi*. Until recently, only a few reports have recorded *X. fastidiosa* infections

in olive and classified the strain as subsp. *multiplex* or denoted it as "Genotype A". In 2013 a severe outbreak of *Xf* occurred in Apulia (southern Italy). Several new foci were discovered in 2014, among which a small one in the province of Lecce, in an area comprising the countryside of Ruffano and some neighbouring villages, which was the object of this investigation. The identification of the *Xf* present in this focus was done by ELISA, using a commercial kit (Loewe, Germany) and by PCR, employing three pairs of species-specific primers i.e. XF1/6, FXYgyr499/RXYgyr907 and HL5/6 which amplify 16S rRNA, *gyrB* and *HL* hypothetical protein genes, respectively. To determine the *Xf* subspecies, genomic DNA was extracted from infected olive trees and amplified. Preliminary studies for single nucleotide polymorphisms (SNPs) assessment on the three above mentioned genes were carried out for strain genotyping. Phylogenetic analyses were conducted using the maximum parsimony method with 1000 bootstrap replicates (MEGA v.6.0 program). Of the three genes investigated, only *gyrB* and *HL* provided sufficient information for assigning the *Xf* strain under study to a known subspecies within a phylogenetic cluster. Nucleotide sequences of the *HL* hypothetical protein gene available in GenBank are very few, however, using this gene it was possible to distinguish two subspecies: *pauca* and *fastidiosa*. By contrast, nucleotide variations in the *gyrB* subunit gene were able to separate *X. fastidiosa* subsp. *pauca* from *multiplex* and *fastidiosa*. Based on these preliminary phylogenetic analyses, the *Xf* strain from the Ruffano focus could be assigned to the subsp. *pauca*, confirming the identification already reported from older foci.

**6. EXTENSIVE LITERATURE SEARCH TO BUILD A DATABASE ON THE HOST RANGE OF *XYLELLA FASTIDIOSA*.** E. Czwienczek<sup>1</sup>, R. Almeida<sup>2</sup>, D. Bosco<sup>3</sup>, G. Stancanelli<sup>1</sup>, J.C. Gregoire<sup>4</sup>, D. Caffier<sup>5</sup>, G. Hollo<sup>1</sup>, O. Mosbach-Schulz<sup>6</sup>, G. Strona<sup>7</sup> and C. Bragard<sup>8</sup>. <sup>1</sup>European Food Safety Authority, ALPHA unit, Plant Health Team, Parma, Italy. <sup>2</sup>Department of Environmental Science, Policy and Management, University of California, Berkeley, USA. <sup>3</sup>Department of Agricultural, Forest and Food Sciences, University of Turin, Turin, Italy. <sup>4</sup>Université Libre de Bruxelles, Belgium. <sup>5</sup>High Council for Biotechnology, France. <sup>6</sup>European Food Safety Authority, AMU unit, Plant Health Team, Parma, Italy. <sup>7</sup>Institute for Environmental Sustainability, Joint Research Centre of the European Commission, Ispra, Italy. <sup>8</sup>Université Catholique de Louvain, Belgium. E-mail: alpha@efsa.europa.eu

Following a request from the European Commission, EFSA was asked to provide: (i) an urgent scientific and technical advice to support emergency measures against the plant pathogenic bacterium *Xylella fastidiosa* (published on EFSA website in autumn 2013); (ii) a complete Pest Risk Assessment (PRA) and evaluation of risk reduction options, which is ongoing and is expected to be completed by November 2014. Thus, a Working Group on *Xylella fastidiosa* (*Xf*) of the EFSA Scientific Panel on Plant Health has been established and is conducting a PRA, keeping also into account up-to-date information on the research and phytosanitary status on the Apulian outbreak on olives. *Xf* was detected in olive trees in the Lecce province (Apulia, southern Italy) in October 2013, this being the first *Xf* outbreak under field conditions in the European Union. The main pathway identified by EFSA in 2013 for the entry and spread of *Xf* is the movement of plants for planting; infective *Xf* vectors transported on plant consignments are also of possible concern. For natural *Xf* spread, the only route is by insect vectors that generally fly short distances up to 100 meters, but can be transported by wind over longer distance. To provide scientific support to the EU risk managers through a PRA, a key element is the review of the host range of the pest. PRA allows to assess the risk associated with entry and spread pathways, and to evaluate the effectiveness of measures to reduce such risk. Known host



plants of *Xf* include many cultivated and spontaneous species that are common in Europe. However a range of European wild plant species would meet this bacterium for the first time, increasing the uncertainty on the host range. Strategies for an extensive literature search and for a standardised data collection on host plants of *Xf* are presented. From the extensive literature search a total of 309 host plant species have been identified up to date, belonging to 63 plant families and 118 genera. The results of the search have been collected into a database, which includes many different aspects (locations, time of collection, sample size, etc.). Maps and summary tables have been compiled based on this database.

**7. XYLELLA FASTIDIOSA IN NATURALLY INFECTED PLANTS IN SOUTHERN APULIA: AN ULTRASTRUCTURAL STUDY.** A. De Stradis<sup>1</sup>, M. Saponari<sup>1</sup>, G. Loconsole<sup>1</sup>, O. Pote-re<sup>2</sup>, D. Boscia<sup>1</sup>, G.P. Martelli<sup>2</sup>. <sup>1</sup>Istituto per la Protezione Sostenibile delle Piante del CNR Sezione Virologia Bari, Via Amendola 165/A, 70126 Bari, Italy. <sup>2</sup>Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy. E-mail: a.destradis@ba.iov.cnr.it

The discovery of *Xylella fastidiosa* (*Xf*) in olive trees affected by the quick decline syndrome (OQDS) in the Salento peninsula of Apulia (southern Italy) had prompted a series of studies which, among other aspects, investigated the presence of the bacterium in the xylem vessels of infected olives. These observations have now been extended to hosts other than olive that showed leaf scorching and desiccations of the canopy. In these hosts (*Acacia saligna*, *Nerium oleander*, *Polygala myrtifolia*, *Prunus amygdalus*) the presence of *X. fastidiosa* had previously been ascertained by PCR and ELISA. Negatively stained leaf dips from all hosts showed the presence of bacterial cells ca. 3 µm in length, whose bodies and fimbriae were clearly decorated by colloidal gold-tagged *Xf*-specific antibodies. The most outstanding cytopathological features were: (i) consistent and massive presence of bacterial cells with rippled cell walls in many xylem vessels of all hosts; (ii) deformed phloem elements with companion cells showing a condensed cytoplasm; (iii) accumulations of electron-dense material of undetermined nature that nearly occluded some xylem vessels.

**8. FLUORESCENCE-BASED PORTABLE FORMAT OF ISOTHERMAL LAMP REACTION FOR DETECTION OF XYLELLA FASTIDIOSA.** T. Dreö, M. Pirc, T. Jakomin, P. Kogovšek and M. Ravnikar. National Institute of Biology, Department of Biotechnology and Systems Biology, Vecna pot 111, SI-1000 Ljubljana, Slovenia. E-mail: tanja.dreö@nib.si

On-site detection of bacterial and other pathogens has become accessible with the development of the loop-mediated isothermal amplification procedure (LAMP) and is of particular value during extensive outbreaks. The results of the LAMP reaction can be assessed in different ways, i.e. turbidimetric measurement, colour change and fluorescence accumulation. Each of these detection approaches has advantages and disadvantages. Fluorescence-based LAMP allows real-time detection and easier interpretation of the results, and incorporates an additional step of melting curve analysis that provides confirmation of the results. The tubes do not have to be opened, minimising the risk of sample contamination. The reaction can be run in laboratory settings in a high-throughput format on a real-time PCR cyclor or in a portable instrument (e.g. Genie, OptiGene, UK). In our study we have transferred a previously described LAMP assay targeting the rimM gene (Harper *et al.*, 2010, *Phytopathology* **100**, 1282-1288) from a colour-change format to a fluorescent-based LAMP assay by incorporating intercalating dyes

into the reaction mixture. This allowed a faster detection of purified DNA of *Xylella fastidiosa* subsp. *multiplex* strain LMG 9063 (ATCC 35871 isolated from *Prunus salicina*) with the highest concentrations detected in nine minutes. Detection was possible down to 80 copies per reaction, corresponding to 10<sup>5</sup> cells/ml of the original sample, in 11-30 min. No spurious amplifications were observed in no template controls, indicating that LAMP is a promising test for on-site detection of *X. fastidiosa* in symptomatic samples. Further studies are underway to assess the influence of crude extracts from olives and other plant material on the analytical sensitivity of the fluorescence-based LAMP for *X. fastidiosa* detection. In testing for other pathogens in grapevines and in environmental sample we have observed that LAMP assays seem to be more resistant to plant inhibitors than real-time PCR, and can thus be run on a cruder sample extracts allowing for a simpler and faster DNA extraction protocols [Kogovšek *et al.*, 2014, *Plant Pathology* **104** (in press); Lenarcic *et al.*, 2014 PLoS ONE **9**, e96027].

**9. INTRA AND INTER-LABORATORY EVALUATION OF MOLECULAR METHODS FOR THE DETECTION OF XYLELLA FASTIDIOSA.** B. Legendre<sup>1</sup>, V. Olivier<sup>1</sup>, D. Molusson<sup>1</sup>, S. Mississipi<sup>2</sup>, A. Couton<sup>3</sup>, M. Hervouet<sup>1</sup>, M. Moisson<sup>1</sup> and F. Poliakov<sup>1</sup>. <sup>1</sup>Plant Health Laboratory, French Agency for Food, Environmental and Occupational Health and Safety - Anses, 7 rue Jean Dixmères, 49044 Angers, France. <sup>2</sup>Nestlé Research and Development Center, 101 Av. Gustave Eiffel, Notre Dame d'Oé, 37097 Tours, France. <sup>3</sup>FREDON des Pays-de-la-Loire, 9, Avenue du Bois L'Abbé, 49071 Beaucouze Cedex, France E-mail: bruno.legendre@anses.fr

*Xylella fastidiosa* (*Xf*) is a plant pathogenic bacterium originating from the Americas. It is now an emergent bacterium in Europe and Middle-East, with an Italian outbreak reported in 2013 on olive tree (Saponari *et al.* 2013, *Journal of Plant Pathology* **95**: 668) and an Iranian outbreak reported in 2014 on grapevine and almond (Amanifar *et al.*, 2014, *Phytopathologia Mediterranea* **53**: 16-25). In 2012, *Xf* was isolated in France by the Plant Health Laboratory (Anses-LSV) from four *Xf*-infected imported coffee plants (*Coffea arabica* and *C. canephora*) growing under containment facilities. *Xf* is a regulated quarantine bacterium for the European Union and, as such, is listed on the directive 2000/29/EC. More than 200 plant species belonging to more than 50 botanical families have been reported as hosts susceptible to *Xf*. Many infected plants could remain symptomless with low-level bacterial concentration. The objective of the present study was to evaluate detection methods based on End-Point PCR and Real-Time PCR in order to select efficient tools for testing asymptomatic imported plants in the framework of official plant protection controls. Several End-Point PCR and one Real-Time PCR protocol were evaluated firstly under an intra-laboratory study, then by seven laboratories under an inter-laboratory study. The following criteria were evaluated: inclusivity, exclusivity, diagnostic sensitivity, diagnostic specificity, repeatability, reproducibility and limit of detection. Tests were performed on various artificially contaminated host material such as coffee (*Coffea arabica* and *C. canephora*), grapevine (*Vitis vinifera*), peach (*Prunus persica*), sweet orange (*Citrus sinensis*) and olive (*Olea europaea*). The DNeasy plant mini kit (Qiagen, USA) was used for DNA extraction. Among the evaluated methods, the best performances were obtained first with the Real-Time PCR (Harper *et al.*, 2010 (erratum 2013), then with the End-Point PCR (Minsavage *et al.*, 1994). In the best conditions, samples contaminated at a level of 3.10<sup>2</sup> bacteria/ml (1.5.10<sup>2</sup> bacteria/g of tissue) could be detected with the Real-Time PCR (Harper *et al.*, 2010). Nevertheless, high variations in detection limit were observed depending on the plant species and sampling method.

**10. MULTILOCUS SEQUENCE TYPING REVEALS THE GENETIC DISTINCTIVENESS OF THE *XYLELLA FASTIDIOSA* STRAIN CoDiRO. G. Loconsole<sup>1</sup>, R. Almeida<sup>2</sup>, D. Boscia<sup>1</sup>, G.P. Martelli<sup>3</sup> and M. Saponari<sup>1</sup>.** <sup>1</sup>Istituto per la Protezione Sostenibile delle Piante del CNR Sezione Virologia Bari, Via Amendola 165/A, 70126 Bari, Italy. <sup>2</sup>Department of Environmental Science, Policy and Management, University of California, Berkeley, CA 94720, USA. <sup>3</sup>Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, Via Amendola 165/A, 70126 - Bari, Italy. E-mail: g.loconsole@ba.ivv.cnr.it

Taxonomically, *Xylella fastidiosa* (*Xf*) constitutes a single species with four recognized subspecies (*Xf. fastidiosa*, *multiplex*, *pauca* and *sandyi*). For identifying subspecies and determining the taxonomic allocation of novel bacterial isolates, a widely used approach is multilocus sequence typing (MLST) analysis that encompasses seven different *loci*. This approach was used for the identification of the *Xf* strain isolated from olive trees affected by the quick decline syndrome (OQDS) in the the Salento peninsula (Apulia, south-eastern Italy). Bacterial DNA recovered from different naturally infected host plants was used to this aim. Specifically, bacterial isolates from six olive trees present in different OQDS foci and one isolate each from oleander, cherry, almond, myrtle-leaved milkwort, and periwinkle were analyzed and compared. MLST analysis showed that the *Xf* strain present in Apulia is taxonomically related to isolates of the subspecies *pauca*, but is distinct from other genotypes of this cluster. Five of the seven MLST *loci*, carrying alleles diagnostic for the South American *Xf pauca* strains infecting citrus and/or coffee, are also present in the Apulian olive isolate. The two remaining *loci* harbour novel alleles, which are closely related with the homologous *loci* of subspecies *pauca*. A novel, hitherto undescribed "Sequence Type" profile (ST53) (<http://pubmlst.org/xfastidiosa/>), was therefore assigned to the Apulian olive isolate based on MLST analysis. Multiple alignments of the DNA sequences of the bacterial isolates recovered from all susceptible hosts analysed, showed a 100% nucleotide identity, indicating that infections are caused by the same genotype. In conclusion, the data so far collected strongly support the notion that the olive isolate of *X. fastidiosa* represents a novel strain within the subspecies *pauca*, for which the name CoDiRO (abbreviation from Complesso del Disseccamento Rapido dell'Olivo, the Italian name of the disease) has been proposed.

**11. USE OF A NEW SNP-BASED ASSAY AND MULTILOCUS SSR MARKERS TO ASSESS THE GENETIC DIVERSITY IN *XYLELLA FASTIDIOSA* subsp. *PAUCA* AND ITS POTENTIAL APPLICATION TO THE STUDY OF THE OLIVE QUICK DECLINE SYNDROME IN ITALY. M. Montes-Borrego<sup>1</sup>, J.R.S. Lopes<sup>2</sup>, R.M. Jiménez-Díaz<sup>1,3</sup>, J.A. Navas-Cortés<sup>1</sup>, G. Loconsole<sup>4</sup>, M. Saponari<sup>4</sup> and B.B. Landa<sup>1</sup>.** <sup>1</sup>Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), 14080 Córdoba, Spain. <sup>2</sup>Departamento de Entomología, Fitopatología e Zoología Agrícola, ESALQ, Universidade de São Paulo, Piracicaba, São Paulo 13400-970, Brazil. <sup>3</sup>College of Agriculture and Forestry, University of Córdoba, 14071 Córdoba, Spain. <sup>4</sup>Istituto per la Protezione Sostenibile delle Piante del CNR, Sezione Virologia Bari, Via Amendola 165/A, 70126 Bari, Italy. E-mail: blanca.landa@csic.es

*Xylella fastidiosa* is a Gram-negative, xylem-inhabiting phytopathogenic bacterium that is insect-transmitted and has very slow growth *in vitro*. This bacterium causes severe diseases and great yield losses in a number of very important crops, including almond, citrus, coffee, grapevine, and peach, and other disorders of perennial crops and landscape plants such as oleander. Several pathogenic variants of the bacterium have been described, which are often host-specific and have been raised taxonomically to the subspecies level: *Xylella fastidiosa* subsp. *fastidiosa*, *X. fastidiosa* subsp.

*multiplex*, *X. fastidiosa* subsp. *pauca*, and *X. fastidiosa* subsp. *sandyi*. We have identified two haplotypes of *X. fastidiosa* subsp. *pauca* (*Xfp*) based in the *gyrB* sequence of more than 90 bacterial isolates infecting citrus and coffee plants in different states in Brazil. The haplotypes were identified based on a single-nucleotide polymorphism (SNP) and correlated with the host of origin. From that SNP, a mini-sequencing protocol was designed to differentiate haplotypes according to the host source that showed robustness for predicting *Xfp* host source in blind assays using DNA extracted from cultures of *Xfp*-infected plants, and bacterial-fed insect vectors. The combined use of the newly developed mini-sequencing protocol and of three previously developed multilocus SSR markers indicated that two haplotypes and distinct isolates of *Xfp* infect citrus and coffee in Brazil, and that multiple isolates genetically different may be present in a single orchard or infecting a single tree. The combined use of the SNP-based protocol and SSR analyses can be very useful for the spatio-temporal epidemiological studies of *Xfp* in Brazil and for the recent outbreak of *X. fastidiosa* in olive in Apulia (Italy) aimed at determining host specificity of bacterial isolates, existence of host jumping, vector feeding habits, etc. Indeed, *X. fastidiosa* isolates from Italy are phylogenetically close to *Xfp* based on *gyrB* sequences and also show a new SNP that differentiates them from the Brazilian citrus and coffee isolates.

**12. SURVEY FOR THE PRESENCE OF *XYLELLA FASTIDIOSA* subsp. *PAUCA* STRAIN CoDiRO IN SOME FORESTRY AND ORNAMENTAL SPECIES IN THE SALENTO PENINSULA. O. Potere<sup>1</sup>, L. Susca<sup>1</sup>, G. Loconsole<sup>2</sup>, M. Saponari<sup>2</sup>, D. Boscia<sup>2</sup>, V. Savino<sup>1</sup> and G.P. Martelli<sup>1</sup>.** <sup>1</sup>Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy. <sup>2</sup>Istituto di Protezione Sostenibile delle Piante del CNR, Sezione Virologia, Bari, Via Amendola 165/A, 70126 Bari, Italy. E-mail: oriana.potere@uniba.it

*Xylella fastidiosa* (*Xf*) is a xylem-inhabiting and vector-transmitted quarantinable bacterium that causes a variety of diseases in a broad range of plant hosts. Recently, the presence of *Xf* was reported in the province of Lecce (south-east Italy) in oleander, almond, cherry and some ornamental species, showing desiccation and scorching of the leaves and, in olive, in which it induces a severe disorder denoted 'quick decline syndrome' (OQDS). The spittlebug *Philaenus spumarius* is the currently ascertained local vector. Due to the fact that: (i) the local strain of *Xf*, denoted CoDiRO has, as expected, a host range that includes several cultivated and wild plants present in Europe, (ii) it is common knowledge that many hosts can carry the pathogen without showing symptoms and (iii) the long-distance dispersal of *Xf* is mediated by the movement of infected plants and vectors present on plant consignments, on February 2014 the European Union has issued the Commission Implementing Decision L 45/29-31 which prohibits the movement from the province of Lecce of all plant genera and species not listed in the Annex I of the cited Decision. This raised the need to prove that certain kinds plants not included in Annex 1 were not hosts of the CoDiRO strain. A survey was therefore carried out in March-April 2014 to verify whether a number of ornamental and forestry plants naturally exposed to inoculum pressure were infected, using the currently available diagnostic protocols. Samples were collected from 207 conifers, 105 Palmaceae and 208 succulent plants (totalling 520) and comparatively examined by molecular (PCR) and serological (DAS-ELISA) methods. A positive ELISA reaction was obtained from 22 of 62 samples of *Pinus*, *Cedrus* and *Libocedrus*. As this result could not be confirmed by repeated PCR rounds, molecular assays were exclusively used for *Xf* detection in conifers. By contrast ELISA and PCR results were in complete agreement for Palmaceae and succulent plants. In conclusion, the totality of the 520 samples of conifers, Palmaceae and succulent plants collected

in the heavily *Xf*-infected area proved to be free from the CoDiRO strain of the bacterium, suggesting that the tested species may not be susceptible to it.

**13. DEVELOPMENT OF AN INFORMATION ACQUISITION SYSTEM FOR THE FIELD MONITORING OF *XYLELLA FASTIDIOSA*.** F. Santoro<sup>1</sup>, G. Favia<sup>1</sup>, F. Valentini<sup>1</sup>, S. Gualano<sup>1</sup>, A. Guarino<sup>2</sup>, A. Percoco<sup>2</sup> and A.M. D'Onghia<sup>1</sup>. <sup>1</sup>CIHEAM - Istituto Agronomico Mediterraneo di Bari. Via Ceglie 23, 70010 Valenzano (BA), Italy. <sup>2</sup>Osservatorio Fitosanitario della Regione Puglia, Lungomare Nazario Sauro 45/47, 70121 Bari, Italy. E-mail: donghia@iamb.it

Field inspections and sampling to monitor quarantine pests are time-consuming and require high accuracy in data acquisition. Information technology tools are important to gather and provide reliable real-time information on plant status and localization. Handheld devices, such as tablets, can now be part of this process. Thanks to the possibilities offered by high-speed wireless networks (LTE) and Android flexibility, an application for field inspections and sampling in the monitoring of *Xylella fastidiosa* was developed in the framework of a project financed by the Apulia Regional Government. The application named XylApp provides inspectors with a regional cartographic grid allowing for a rapid identification of the site to be monitored and a fast field data storage device, which can instantly record all plant and geo-localization parameters thanks to built-in sensors. Data collected using tablets are sent online to a central server for permanent storage and further processing. Results are used for field or territorial analyses through a software handling data (decision support system), which also gets a visual feedback since this project includes the positioning of georeferenced samples on a browser-based map.

**14. PRELIMINARY RESULTS OF A SURVEY OF WEEDS AS POTENTIAL HOSTS OF *XYLELLA FASTIDIOSA* STRAIN CoDiRO.** L. Susca<sup>1</sup>, O. Potere<sup>1</sup>, S. Marullo<sup>1</sup>, V. Savino<sup>1</sup>, P. Venetito<sup>3</sup>, G. Loconsole<sup>2</sup>, M. Saponari<sup>2</sup>, D. Boscia<sup>2</sup> and P. La Notte<sup>2</sup>. <sup>1</sup>Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy. <sup>2</sup>Istituto di Protezione Sostenibile delle Piante del CNR, Sezione Virologia Bari, Via Amendola 165/A, 70126 Bari, Italy. <sup>3</sup>Centro di Ricerca, Sperimentazione e Formazione in Agricoltura "Basile-Caramia", Via Cisternino, 281, 70010 Locorotondo, Italy. E-mail: p.lanotte@ba.ivr.cnr.it

To investigate the role of herbaceous plants as hosts of *Xylella fastidiosa*, monthly sampling of the native flora of two heavily infected olive groves and of the sides of the adjacent dirt roads was conducted from January 2014 onwards. One of the orchards had not been subjected to weeding, whereas periodic tillage had been carried out in the other. Overall, more than 100 species of 40 monocotyledonous and dicotyledonous families were collected, photographed and identified, their phenological stage was recorded using the Keller-Baggiolini scale, their period of presence and the type of distribution prevailing in the field was assessed (e.g. whether

the species were scattered or concentrated under olive trees or along the edges of the dry stone walls) as well as the abundance-dominance indices according to the Braun-Blanquet method. At all sites monitored, *Philaenus spumarius*, the main vector of *X. fastidiosa* so far found in the area of the outbreak, was present with abundant populations on herbaceous species in the spring and on olive trees from the middle of May throughout the summer. All weed samples collected, in pools of 4-5 plants, were tested by DAS-ELISA and the uncertain results were verified by PCR. So far, none of the samples analyzed, in excess of 600, proved to host *X. fastidiosa*, confirming the preliminary observations that, by and large, weeds may not have a major role in the epidemiology of *X. fastidiosa* in the considered area. However, since sampling of the summer/autumn flora is yet to be done, a better insight into the epidemiological role of these plants will become available in the coming months, in conjunction with the migration of the vectors from the olive trees onto the native flora, with the consequent possible inoculation of the bacterium.

**15. RECENTLY DEVELOPED METHODS FOR *IN SITU* DETECTION OF *XYLELLA FASTIDIOSA* IN OLIVE TREES AND INSECTS.** T. Yaseen, K. Djelouah, F. Valentini, T. Elbeaino, D. Frasheri, M. Digiaro and A.M. D'Onghia. CIHEAM Istituto Agronomico Mediterraneo di Bari. Via Ceglie 23, 70010, Valenzano (Bari) Italy. E-mail: y.thaer@iamb.it

*Xylella fastidiosa*, which is associated with the Olive quick decline syndrome (OQDS), has already infected numerous host species in a large area of Apulia (Southern Italy) and is being rapidly spread by insect vectors. Recent investigations have reported that *Philaenus spumarius* is a recognized vector of the pathogen, while *Neophilaenus campestris* and *Euscelis lineolatus* can also carry by the bacterium but were not proven to be vectors. Nevertheless, the role of these insects can be fundamental when used as "spy insects" for *X. fastidiosa* monitoring as they may reveal its presence in pathogen-free areas before symptoms develop in plants. ELISA and PCR are currently used for the detection of the bacterium in laboratories which may be located outside of the infected area. Direct tissue blot immunoassay (DTBIA) and real-time Loop-mediated isothermal amplification (RT-LAMP) have recently been developed for the detection of *X. fastidiosa* in olive trees, showing the same efficiency of ELISA and PCR with the advantages of easier handling, higher speed and lower cost. RT-LAMP may also detect the pathogen in the 'spy insects'. Both techniques can easily be applied for *in situ* detection of *X. fastidiosa*, avoiding the risk of moving infected plant materials and vectors for analyses. For DTBIA, a 0.45 µm nitrocellulose membrane, 1% fat milk solution instead of BSA, and commercial polyclonal antibodies to *X. fastidiosa* have been used. As to RT-LAMP, newly designed primers and a Smart-Dart have been used to detect the pathogen in plant materials and insects. The fast (10 min) and non-destructive DNA extraction method allows pathogen detection in insects, which can be used for identification after analysis. Due to the uneven distribution of the pathogen in plants, a composite sample prepared with twigs collected from different sides of the canopy is preferred for the analysis, to reduce the risk of false negatives.

