INTERNATIONAL SYMPOSIUM ON THE EUROPEAN OUTBREAK OF XYLELLA FASTIDIOSA IN OLIVE

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ORAL PRESENTATIONS

OCCURRENCE OF *XYLELLA FASTIDIOSA* IN APULIA. **D. Boscia.** *Istituto per la Protezione Sostenibile delle Piante (former Istituto di Virologia Vegetale) del CNR, UOS Bari, Via Amendola 122/D, 70126 Bari, Italy. E-mail: d.boscia@ba.ivv.cnr.it*

A strain of *Xylella fastidiosa* subsp. *pauca* denoted CoDiRO (abbreviation from the Italian name "Complesso 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 preoccupantly 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. **D. Bosco[1], R. Almeida[2], E. Czwienczek[3], G. Stancanelli[3], J. C. Gregoire[4], D. Caffier[5], G. Hollo[3], C. Bragard[6].** *[1]Department of Agricultural, Forest and Food Sciences, University of Turin, Turin, Italy.[2]Department of Environmental Science, Policy and Management, University of California, Berkeley, USA. [3]European Food Safety Authority, ALPHA Unit, Plant Health Team, Parma, Italy. [4]Université Libre de Bruxelles, Belgium. [5]High Council for biotechnology, France. [6]Université Catholique de Louvain, Belgium. E-mail: domenico.bosco@unito.it*

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 loose 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. **G.H. Cardon.** *European Commission, Health and Consumers Directorate-General. E-mail:guillermo.cardon@ec.europa.eu*

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. **H.D. Coletta Filho,** *Centro de Citricultura Sylvio Moreia, Instituto Agronomico de Campinas, 13490-000 Cordeiropolis, SP, Brazil. E-mail: helvecio@centrodecitricultura.br*

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[1], G. Loconsole[2], D. Boscia[2], A. De Stradis[2], R. K. Yokomi[3], D. Bosco[4], F. Porcelli[1], G. P. Martelli[1] and M. Saponari[2]. *[1]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy. [2]Istituto per la Protezione Sostenibile delle Piante del CNR (former Istituto di Virologia Vegetale), UOS Bari, Via Amendola 122/D 70126 Bari, Italy. [3]United States Department of Agriculture-Agricultural Research Service (USDA-ARS), Crop Diseases, Pests and Genetics Research Unit, 9611 S. Riverbend Ave., Parlier, CA 93648, USA. [4]Dipartimento di Scienze Agrarie, Forestali e Alimentari, Università degli Studi di Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. E-mail: francesco-porcelli@uniba.it*

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 it 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: *PHILAENUS 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 autum-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 Cordeiropolis, 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 causes 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.

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AN INNOVATIVE MONITORING MODEL FOR *XYLELLA FASTIDIOSA* IN APULIA. A.M. D'Onghia[1], F. Santoro[1], T. Yaseen[1], K. Djelouah[1], A. Guario[2], A. Percoco[2], T. Caroppo[3] and F. Valentini[1]. *[1]CIHEAM, Istituto Agronomico Mediterraneo di Bari. Via Ceglie 23, 70010, Valenzano (Bari), Italy. [2]Osservatorio Fitosanitario della Regione Puglia, Lungomare Nazario Sauro 45/47, 70121 Bari, Italy. [3]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[1], M. Chiumenti[1], M. Saponari[1], G. Donvito[2], A. Italiano[2], G. Loconsole[1], C. Cariddi[3], G.P. Martelli[3] and P. Saldarelli[1]. *[1]Istituto per la Protezione Sostenibile delle Piante del CNR (former Istituto di Virologia Vegetale), UOS Bari, Via Amendola 122/D, 70126 Bari, Italy. [2]Istituto Nazionale di Fisica Nucleare, Via Orabona, 4, 70125 Bari, Italy. [3]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.ivv.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). 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: dhop@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[1], S. Mississipi[1,2], V. Olivier[1], E. Morel[2], D. Crouzillat[2], K. Durand[3], P. Portier[3], F. Poliakoff[1], and M. A. Jacques[3]. *[1]Anses, Plant Health Laboratory, 7 rue Jean Dixméras, 49044 Angers, France. [2]Nestlé Research and Development Center, 101 Av. Gustave Eiffel, Notre Dame d'Oé, 37097 Tours, France. [3]INRA, UMR1345, IRHS, 42 rue Georges Morel, 49071 Beaucouzé, 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 (EPPO 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[1*], O. Potere[2*], T. Elbeaino[3], D. Frasheri[3], S. Frisullo[4], F. Palmisano[5], D. Boscia[1] and M. Saponari[1]. *[1]CNR Istituto di Protezione Sostenibile delle Piante del CRN (former Istituto di Virologia Vegetale) UOS Bari, Via Amendola 122/D, 70126 Bari, Italy. [2]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy. [3]Istituto Agronomico Mediterraneo, Via Ceglie 9, 70100 Valenzano (BA), Italy. [4]Dipartimento di Scienze Agrarie, degli Alimenti e dell'Ambiente, Università degli Studi, Via Napoli 25, 71100 Foggia, Italy. [5]Centro di Ricerca, Sperimentazione e Formazione in Agricoltura, Via Cisternino 281, 70100 Locorotondo (Bari), Italy. E-mail: g.loconsole@ba.ivv.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^{-5}, while qPCR was 10^2 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 implelented 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 booth 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 periwikle 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 medow spittlebug (*P. spumarious*) 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 froghopper 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 occurr 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, β-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 *Neofusiccoccum mediterraneum*, *N. australe*, and *N. vitifusiforme*, both in discolored sapwood and in

cankered bark. Isolation of *Neofusicoccum* spp. from sapwood of declining trees outside the *Xylella*-infected areas was sporadic. Fungal isolates morphologically similar to *Phaeomoniella* 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. Notwhistanding the morphological similarity with the yeast-like culture of *Phaeomoniella*, this fungus should be regarded as an undescribed species belonging to the *Coelomycetes* 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@eppo.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: ahpurcell@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 europeae*) 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 he 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[1], G. Loconsole[1], R. Almeida[2], H.D. Coletta-Filho[3], G.P. Martelli[4] and D. Boscia[1].** *[1]Istituto di Protezione Sostenibile delle Piante del CNR, Sezione di Bari Via Amendola 165/A, 70126 Bari, Italy. [2]Department of Environmental Science, Policy, and Management, UC Berkeley. California, USA. [3]Centro de Citricultura Sylvio Moreira IAC, Cordeiropolis, SP, Brazil. [4]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.ivv.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, discolouration 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[1], R. Almeida[2], D. Bosco[3], C. Bragard[4], D. Caffier[5], J. C. Gregoire[6], S. Parnell[7], G. Strona[8], O. Mosbach-Schulz[9], E. Czwienczek[1], G. Hollo[1].** *European Food Safety Authority, ALPHA unit, Plant Health Team, Parma, Italy. [2]Department of Environmental Science, Policy and Management, University of California, Berkeley, USA. [3]Department of Agricultural, Forest and Food Sciences, University of Turin, Turin, Italy. [4]Université Catholique de Louvain, Belgium. [5]High Council for Biotechnology, France. [7]Rothamsted Research, Harpenden, United Kingdom. [8]Institute for Environmental Sustainability, Joint Research Centre of the European Commission, Ispra; Italy. [9]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[1,2], J.M. Bullock[1], D.A.P. Hooftman[1], D.S. Chapman[3].** *[1]Centre for Ecology and Hydrology, Benson Lane, Wallingford, Oxfordshire, OX10 8BB, UK. [2]Mathematical Institute, University of Oxford, Andrew Wiles Building, Radcliffe Observatory Quarter, Woodstock Road, Oxford, Oxfordshire, OX2 6GG, UK. [3]Centre for Ecology and Hydrology, Bush Estate, Penicuik, Midlothian, EH26 0QB, UK. E-mail: steven.white@ceh.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.