with the "Olive Quick Decline Syndrome" (OQDS) widespread in the Salento area (Southern Italy). The rapid emergence of this regulated quarantine pathogen in new areas imposes the application of official diagnostic methods aimed to prevent it's spread in other Italian regions and the movement of latently infected plant material. The present study summarizes the results of a test-performance study (TPS) to validate, at a national level, detection methods of *X. fastidiosa* by involving sixteen Italian laboratories that includes several Plant Protection Services (PPS), SELGE, UniMi, UniCT, CRSFA, CIHEAM. A working group was constituted, that organized: i) a PRE-TEST for the establishment of analytical-sensitivity of each method and repeatability, analytical-specificity, relative accuracy, ii) the final TPS to detect the reproducibility of the selected methods. The activity of the pre-test highlighted a higher analytical-sensitivity from samples of total-DNA with respect crude-extracts. In particular, LAMP-PCR was more sensitive than ELISA tests from crude-extracts. Using total-DNA, duplex (based on *rimM* and on *cox* primers) and single real-time PCR (*rimM* primers) resulted in the most sensitive methods followed by LAMP-PCR and, finally, conventional PCR (primers RST31/RST33). The high values of relative-accuracy and reproducibility (among 92-100% for both) confirmed a high reliability of duplex/single real-time PCRs and LAMP-PCR from total-DNA. LAMP-PCR from crude extracts gave values of accuracy and reproducibility respectively of 78% and 87% resulting a promising assay for its friendly and on-site-based use, thus implementing phytosanitary inspections prior to import/export of plant material and controls in orchards or in nurseries.

A GENOME-WIDE APPROACH TO REDEFINE *XYLELLA FASTIDIOSA* TAXONOMY.

S. Marcelletti[1], M. Scortichini[1,2]. *[1]CREA-Centro di ricerca per le Colture Arboree, Via di Fioranello, 52 - 00134 Roma, Italy. [2]CREA-Centro di ricerca per le Colture Arboree, Via Torrino, 3 - 81100 Caserta, Italy. E-mail: marco.scortichini@crea.gov.it*

Xylella fastidiosa is a xylem-limited, fastidious phytopathogenic bacterium of the *Xanthomonadaceae* family which colonizes a very large number of hosts. Recently, *X. fastidiosa* strains belonging to the subspp. *pauca* and *multiplex* have been isolated in Southern Europe from *Olea europea* (Salento area; Southern Italy) and several ornamental shrubs (Corsica, Maritime Alps; Southern France). The rapid emergence of this regulated phytopathogen in new areas imposes the application of rapid and reliable detection techniques to prevent the further introduction of latently infected plant material. The knowledge of the pathogen basic taxonomy and population structure is fundamental for the development of an efficient detection and prevention protocol. The incorporation of genomic data into bacterial taxonomies and systematic procedures has recently greatly contributed to the advancement of such disciplines. A total of 21 *Xylella fastidiosa* strains were assessed by comparing their genomes to infer their taxonomic relationships. The whole-genome-based average nucleotide identity (ANI) and tetranucleotide frequency correlation coefficient (TETRA) analyses were performed. In addition, a consensus tree based on comparisons of 956 core gene families, a genome-wide phylogenetic tree and a neighbor-network were constructed with 820.088 nucleotides (i.e., approximately 30-33% of the entire *X. fastidiosa* genome). All approaches revealed the occurrence of three well demarcated genetic clusters that represent *X. fastidiosa* subspecies, namely *fastidiosa*, *multiplex* and *pauca*. Moreover, the proposed but never formally described subspecies '*sandyi*' and '*morus*' are instead members of the subspecies *fastidiosa*. These analyses also revealed the existence of a new *Xylella* species that was isolated in Taiwan from *Pyrus pyrifolia*.

A METAGENOMIC INVESTIGATION OF THE MICROBIOME OF *XYLELLA FASTIDIOSA*-INFECTED OLIVES.

A. Giampetruzzi[1], M. Saponari[2], G. D'Attoma[1,2], M. Morelli[2], M. Chiumenti[2], D. Boscia[2], P. Saldarelli[2]. *[1]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti (Di.S.S.P.A.) Università degli Studi di Bari Aldo Moro - 70126 Bari. [2]CNR Istituto per la Protezione Sostenibile delle Piante (IPSP), SS Bari - 70126 Bari, Italy. E-mail: pasquale.saldarelli@ipsp.cnr.it*

Following the introduction and establishment of the plant pathogenic bacterium *Xylella fastidiosa* (*Xf*) in the Apulia Region (southern Italy), olive turned to be the main host of the Salentinian bacterial strain and the majorly devastated crop. The mechanism of pathogenicity of *Xf* is still not completely understood and no means to cure the bacterium in the infected plants are available yet. Nevertheless, the alteration of microbial communities and effects in the expression of symptoms of *Xf*-infected plants is poorly studied. We are investigating the microbiome of *Xf*-infected olives by

a shotgun metagenomic DNA sequencing approach that avoids the limitations of amplicon sequencing. Data obtained (28,333,924 and 29,096,610 reads from *Xf*-infected and healthy plants) were analyzed by MetaPhlAn, a metagenomic abundance estimation tool which maps reads to a set of selected marker sequences. Libraries from xylem tissues revealed a complex community in which small symbiotic bacteria of insects, i.e. *Candidatus* Zinderia insecticola and *Candidatus* Carsonella ruddii represented the 31% and 22% of the total population. *Xf* reaches in infected plants the 12% of the total microbial community. Studies are ongoing to characterize the microbial communities in the xylem sap of tolerant and susceptible olive cultivars, to envisage a control strategy based on the manipulation of these resident communities and to identify endosymbiont(s) which may be used to reduce the severity of symptoms. To this end, the evaluation of an endosymbiont bacterium for its potential to colonize *Xf*-infected olive tissues is underway.

RAPID SCREENING TESTS FOR DIFFERENTIATING *XYLELLA FASTIDIOSA* ISOLATES.

M. Saponari[1], M. Montes-Borrego[2], G. D'Attoma[1,3], L. De La Fuente[4], G. Loconsole[3], B.B. Landa[2]. *[1]CNR Istituto per la Protezione Sostenibile delle Piante UOS Bari (IPSP), SS Bari, 70126 Bari, Italy. [2]CSIC Instituto de Agricultura Sostenible, 14080 Córdoba, Spain. [3]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti (Di.S.S.P.A.) Università degli Studi di Bari Aldo Moro, 70126 Bari. [4]Department of Entomology and Plant Pathology Auburn University, 36849 Auburn, AL, USA. E-mail: maria.saponari@ipsp.cnr.it*

The bacterial pathogen *Xylella fastidiosa* (*Xf*) is characterized by a wide plant host range and insect vectors, and on the basis of phylogenetic studies it was subdivided into different subspecies. Results from strain typing, phylogenetic analyses, and other data comparisons have shown that phylogenetic clusters exhibit host-based genetic relationships. Until now, different molecular tests can be used for the differentiation of *Xf* isolates, among which MLST/MLSA represents the most common method to determine classification and phylogenetic placement of novel isolates. *Xf* outbreaks in EU motivated the search for accurate and faster approaches for detection and identification of the bacterium in different plant matrices. Because MLST/MLSA requires several PCR reactions and sequencing analyses, we have developed two independent approaches for rapid taxonomic assignment of uncharacterized isolates: (1) single-nucleotide primer extension (SNuPE) method for the multiplex amplification of six *Xylella* DNA sequences (targeting all subspecies and three genotypes within *Xf* subsp. *pauca* including the type-isolate infecting olive in Italy); (2) high-resolution melting analysis of the amplicon recovered from the gene encoding the conserved HL protein. Both assays proved to clearly differentiate *Xf* isolates currently known to occur in the Italian and France outbreaks. Indeed, validation on a larger panel of isolates covering the different subspecies consistently allowed to rapidly differentiate the isolates in different clusters. In conclusion, these approaches represent a useful tool for pre-screening and selection of infected samples to be further analyzed by MLST or whole genome sequencing.

TRANSMISSION OF *XYLELLA FASTIDIOSA* TO DIFFERENT HOST PLANTS BY NATURALLY INFECTED *PHILAENUS SPUMARIUS*.

V. Cavalieri[1], D. Cornara[2], C. Dongiovanni[3], G. Altamura[1], D. Boscia[1], F. Porcelli[2], D. Bosco[4], M. Saponari[1]. *[1]CNR Istituto per la Protezione Sostenibile delle Piante (IPSP), SS Bari, 70126 Bari, Italy. [2]Centro di Ricerca, Sperimentazione e Formazione in Agricoltura (CRSFA) "Basile Caramia", 70010 Locorotondo (Bari), Italy. [3]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti (Di.S.S.P.A.) Università degli Studi di Bari Aldo Moro, 70126 Bari. [4]Università degli Studi di Torino, DISAFA, Grugliasco, (TO) Italy. E-mail: vincenzo.cavalieri@ipsp.cnr.it*

The meadow spittlebug *Philaenus spumarius* (Hemiptera, Aphrophoridae) has been identified as a vector of *Xylella fastidiosa* (*Xf*) in southern Italy where the bacterium has established in the Salentinian Peninsula. This species is one of the most common potential vectors in Europe, but limited information is available on spittlebugs as vectors of *Xf*. In this work, eleven transmission experiments were performed in 2015 from late spring to late autumn, when adult spittlebugs were present in the *Xf*-infected olive groves. Insects were collected by sweeping net on the olive canopies of two selected *Xf*-infected olive groves and transferred in groups of five on to the following recipient plants: olive, oleander, citrus, grapevine, GF677 (*Prunus persica* x *Prunus amygdalus*) and periwinkle. Following an inoculation access period (IAP) of 7-days, the insects were recovered from the cages in order to estimate i) the survival rates ii) the presence of *Xf* by real-time qPCR in single insects. Transmissions were determined by testing with qPCR the recipient plants. The results showed that the proportion