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

of *P. spumarius* that tested positive for *Xf* ranged from 25 to 71%. *P. spumarius* transmitted *Xf* to all the recipient plants except grapevine; however, citrus and stone fruit plants were not systemically infected. More than 75% of the insects survived the 7-day IAP on olive, grapevine, GF677 and periwinkle. A lower survival rate was recorded on citrus and on oleander. These data show that field-collected *P. spumarius* in the Salentinian olive groves have high rates of *X. fastidiosa* and are able to transmit the bacterium to different hosts.

ESTABLISHMENT OF AN EXPERIMENTAL FIELD TO EXPLORE THE DIFFERENTIAL OLIVE CULTIVAR RESPONSE TO *XYLELLA FASTIDIOSA* INFECTION.

M. Saponari[1], F. Palmisano[2], C. Dongiovanni[2], V. Cavalieri[1], G. Altamura[1], G. D'Attoma[1,3], G. Loconsole[3], M. Morelli[1], A. Saponari[2], D. Tavano[1], S. Zicca[1], D. Boscia[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. E-mail: maria.saponari@ipsp.cnr.it*

While different sources of natural resistance to *Xylella fastidiosa* (*Xf*) have been described in grapevines and citrus, lack of consolidated information exists on the wide panel of cultivars characterizing the vast olive germplasm. Preliminary observations on few cultivars, support the evidence that differential cultivar responses to *Xf* infections may exist. To explore the response of a larger panel of cultivars, in April 2015, an experimental olive plot, located within the *Xf*-heavily affected olive groves, was established in the Apulia Region (Italy). Twenty-four trees for each of the ten different cultivars were planted in randomized blocks. Each tree was caged with 15-20 specimens of *Philaenus spumarius* collected from the neighboring infected olive groves. Upon removing the cages, the trees are then continuously exposed to the natural vector populations occurring in the area. Nine and 12-months after planting, the trees were sampled, tested for *Xf* and inspected for symptoms. The first data confirmed the infectivity of the vector populations occurring in the Apulian contaminated area and the *Xf* susceptibility of the olive cultivars tested. Almost 50% of the trees tested positive, with an infection incidence ranging from 25% (Leccino) to 78% (Koroneiki). Symptoms of shoot dieback started to appear 1-year after planting, limitedly on few replicates of Cellina di Nardò. In April 2016, the number of cultivars has been increased up to 30. Periodical surveys for symptoms and quantitative analyses to monitor the differential bacterial titer and expression of target genes involved in the host response, are underway.

REPORT OF '*CANDIDATUS* LIBERIBACTER SOLANACEARUM' IN COMMERCIAL APIACEAE SEEDS IN ITALY.

V. Ilardi [1], E. Di Nicola[1],V. Lumia[1], M. Tavazza[2]. *[1]Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di ricerca per la patologia vegetale (CREA-PAV), via C. G. Bertero, 22 - 00156 Roma, Italy. [2] ENEA, SSPT-BIOAG, Via Anguillarese 301 - 00123- Rome, Italy. E-mail: vincenza.ilardi@crea.gov.it*

'*Candidatus* Liberibacter solanacearum' (CaLsol) has been recently shown to be seed-borne in carrot (*Daucus carota*), family Apiaceae. Therefore, exclusion of infected seedlots is necessary to prevent its introduction in new areas, such as Italy. Here, we tested seedlots of five carrot varieties sold in Italy during 2015 for CaLsol by i) real-time PCR assay (rtPCR) of the 16S rRNA locus; ii) PCR assay of the intergenic region between the 16S and 23S rRNA genes (ISR16/23S); and iii) PCR assay of the 50S rpIJ/rpIL ribosomal protein genes (50SrpIJ/L). CaLsol DNA was detected in seedlots of four varieties regardless of the method used. Sequence analysis of PCR-derived 50SrpIJ/L DNA and ISR16/23S DNA amplicons identified two homogeneous groups of CaLsol isolates. The single-nucleotide polymorphism analysis revealed that the first group was closely related to the CaLsol haplotype-E (except for having A at nucleotides 1620 and 1632 of ISR16/23S), while the second to the haplotype-D (except for having A at nucleotide 1648 of ISR16/23S, and T at nucleotides 920 and 1068 of 50SrpIJ/L). The identification of CaLsol in carrot seeds prompted us to investigate its presence in seedlots of another Apiaceae, the parsley (*Petroselium crispum*). Of note, rtPCR and PCR analyses identified CaLsol in seedlots of all three parsley varieties analyzed. Our data indicate that CaLsol-infected seedlots of different carrot and parsley varieties are commercialized in Italy, a country where the presence of CaLsol has not been reported yet, thus highlighting the requirement of coordinated and harmonized measure to limit its spread.

COMPARATIVE STUDY AMONG *NEOFABRAEA* SPP. ITALIAN ISOLATES. I.

Cameldi, F. Neri, M. Meneghini, I. M. Nanni, M.