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Infectivity and Transmission of *Xylella fastidiosa* by *Philaenus spumarius* (Hemiptera: Aphrophoridae) in Apulia, Italy

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ABSTRACT Discovery of *Xylella fastidiosa* from olive trees with “Olive quick decline syndrome” in October 2013 on the west coast of the Salento Peninsula prompted an immediate search for insect vectors of the bacterium. The dominant xylem-fluid feeding hemipteran collected in olive orchards during a 3-mo survey was the meadow spittlebug, *Philaenus spumarius* (L.) (Hemiptera: Aphrophoridae). Adult *P. spumarius*, collected in November 2013 from ground vegetation in *X. fastidiosa*-infected olive orchards, were 67% (40 out of 60) positive for *X. fastidiosa* by polymerase chain reaction (PCR) assays. *Euscelis lineolatus* Brullé were also collected but tested negative for the pathogen. Transmission tests with *P. spumarius* collected from the Salento area were, therefore, conducted. After a 96-h inoculation access period with 8 to 10 insects per plant and a 30-d incubation period, PCR results showed *P. spumarius* transmitted *X. fastidiosa* to two of five periwinkle plants but not to the seven olive plants. Sequences of PCR products from infected periwinkle were identical with those from *X. fastidiosa*-infected field trees. These data showed *P. spumarius* as a vector of *X. fastidiosa* strain infecting olive trees in the Salento Peninsula, Italy.

KEY WORDS spittlebug, vector transmission, xylem-fluid feeding insect, olive

Xylella fastidiosa is a xylem-limited gram-negative bacteria that causes economically important diseases, including Pierce’s disease of grapevine; leaf scorch of almond, oleander, and coffee; citrus variegated chlorosis; and other diseases of crop, forest, and landscape plants (Janse and Obradovic 2010). *Xylella fastidiosa* is well-distributed in the Americas and has been reported from Taiwan causing disease in Chinese pear (*Pyrus pyrifolia* (Burman F.) Nakei) (Leu and Su 1993) and grapevines (Su et al. 2013). In 2010, olive trees on the west coast of Salento Peninsula, Italy, began to decline and die with a condition of unknown etiology called “Olive quick decline syndrome” (OQDS; Nigro et al. 2014; Fig. 1). By 2013, the affected area had grown to ≈10,000 ha. Saponari et al. (2013)

showed all symptomatic trees tested were positive for *X. fastidiosa* DNA by polymerase chain reaction (PCR). Although the role of *X. fastidiosa* in the etiology of this new olive disease has not yet been determined, this was the first widespread detection of the bacterium in Europe and a quarantine around the infected area was imposed (European Food Safety Authority [EFSA] 2013). The status of the Pierce’s disease in grapevines in Kosova following a report by Berisha et al. (1998) is unknown.

X. fastidiosa is transmitted by xylem fluid-feeding hemipterans belonging to Auchenorrhyncha, including cicadellids, and aphrophorids (cercopids; Frazier and Freitag 1946, Hewitt et al. 1946, Severin 1947, Redak et al. 2004). Most known Western Hemisphere *X. fastidiosa* vectors do not occur in Europe. In a recent review of *X. fastidiosa* in Europe, the green leafhopper, *Cicadella viridis* L. (Hemiptera: Cicadellidae) and the meadow spittlebug, *Philaenus spumarius* L. (Hemiptera: Aphrophoridae) were reported as potential vectors (Janse and Obradovic 2010). Several species of *Philaenus* had been previously reported as vectors of *X. fastidiosa* in the United States, including *P. spumarius* (Severin 1950, Purcell 1980). However, Purcell (1989) noted any xylem fluid-feeding hemipteran should be regarded as a potential vector of the bacterium.

Little is known about *C. viridis* or *P. spumarius* in Italy. Therefore, a survey of hemipterans occurring in

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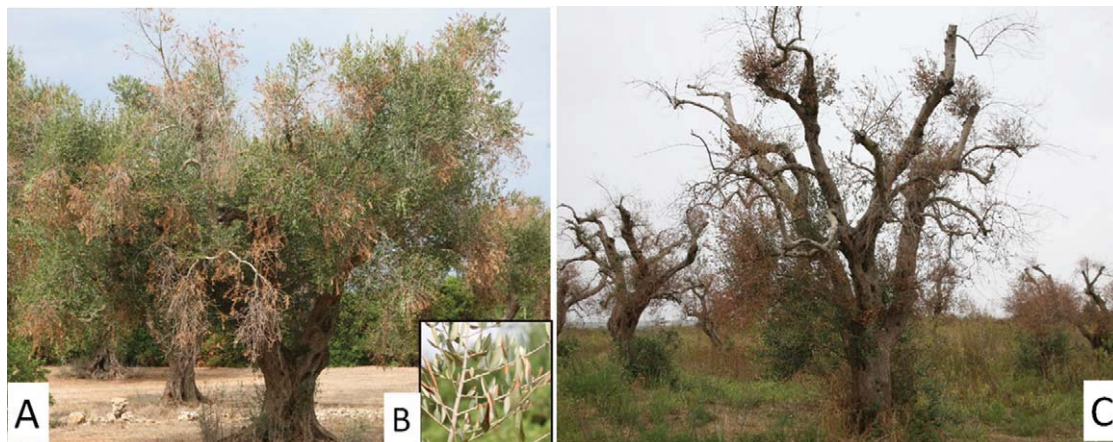


Fig. 1. Olive trees with OQDS on the west coast of the Salento Peninsula, Italy. (A) Branch dieback scattered throughout canopy. (B) Close-up of scorched leaves. (C) Dead or dying trees. (Online figure in color.)

olive groves in the Salento Peninsula was initiated in November 2013. During a 90-d survey, *P. spumarius* was collected and found to harbor *X. fastidiosa*. Transmission tests using field-collected insects showed that *P. spumarius* transmitted the *X. fastidiosa* Salento strain.

Materials and Methods

Sampling Candidate Insect Vectors in Salento Peninsula. From November 2013 through January 2014, ground vegetation (graminaceous, brassicaceous, and thistle weeds) adjacent to and under olive trees, bushes, and ornamentals, were sampled at 2-wk intervals by sweep net to identify the insect population. Six different fields were sampled on the basis of OQDS symptoms and confirmed *X. fastidiosa* infections. Each sample site was swept over a 20 m² area and an average of one *P. spumarius* every 2 m² was obtained. Insects were identified by morphological characters and slide-mounts of genitalia (Le Quesne 1969).

Prevalence of *X. fastidiosa* in Field-Collected Insects. After identification, insects from *X. fastidiosa*-infected olive orchards were analyzed individually by PCR for presence or absence of *X. fastidiosa*. Samples included 160 adult *P. spumarius* and 30 of *Euscelis lineolatus* Brullé (Hemiptera: Cicadellidae) from OQDS-symptomatic olive orchards and 10 *P. spumarius* from a noninfected olive grove in Bari Province (200 km north of the diseased area) as negative controls. Briefly, the head of each insect was dissected from the body and ground in cetyl trimethylammonium bromide-based buffer (Marzachi et al. 1998), and PCR (Loconsole et al. 2014) conducted using 2 µl of the recovered total nucleic acid per PCR reaction with primers X.fas-0838-a-S-21-X.fas-1439-a-A-19 and FXYgyr499-RXYgyr907 (Rodrigues et al. 2003).

Transmission of *X. fastidiosa* by *P. spumarius*. Adult *P. spumarius* (Fig. 2B) collected from infected orchards were caged in groups of 8 to 10 insects per test plant for a 96-h inoculation access period (IAP; Fig. 2A). Test plants were 2-mo-old periwinkle, *Catharan-*

thus roseus G. Don, and 1-yr-old olive, *Olea europea* L. Five periwinkle and seven olive plants were exposed to insects and maintained during IAP and incubation periods in a growth chamber at 26–28°C with a photoperiod of 16:8 (L:D) h. Healthy control plants consisted of two periwinkles and two olives maintained under the same test conditions. Test plants were assayed three times for *X. fastidiosa* at 30-d intervals by PCR (Loconsole et al. 2014). Leaf dips from plants testing positive for *X. fastidiosa* were further subjected to electron microscopy examination for presence of the bacterium. The 602- and 408-bp PCR products from two *X. fastidiosa*-positive *P. spumarius* were sequenced and analyzed by BLAST search and pairwise alignment with *X. fastidiosa* sequences previously identified from OQDS-affected olive trees in the Salento Peninsula (Saponari et al. 2013).

Results

Identification of Insects. In total, ≈415 putative xylem-fluid feeding Auchenorrhyncha were counted in

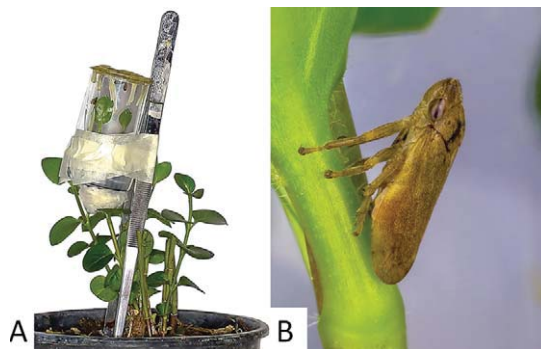


Fig. 2. Adult *P. spumarius* on periwinkle during the IAP for transmission of *X. fastidiosa*. (A) Transparent cage used in the transmission experiments. (B) Close-up of the *P. spumarius*. (Online figure in color.)

Table 1. PCR assays to detect *X. fastidiosa* from xylem fluid-feeding insects collected from olive orchards symptomatic for OQDS in the Salento Peninsula, Italy

Collection date	Species	No. of insects tested by PCR	No. of insects harboring <i>X. fastidiosa</i>
4 Nov. 2013	<i>P. spumarius</i>	40	28
15 Nov. 2013	<i>P. spumarius</i>	20	12
5 Dec. 2013	<i>P. spumarius</i>	20	0
20 Dec. 2013	<i>P. spumarius</i>	30	0
	<i>E. lineolatus</i>	30	0
9 Jan. 2014	<i>P. spumarius</i>	10	0
20 Jan. 2014	<i>P. spumarius</i>	10	0
Total		160 ^a	40

^a In addition, 10 adult *P. spumarius*, collected as controls from nonaffected olive orchards in Bari Province, tested negative for the bacterium.

the sweep-net samples. The most common species was *P. spumarius* comprising 60% (\approx 250 specimens). *E. lineolatus* constituted 30% (\approx 125 specimens) and the remaining 10% (\approx 40 specimens) were unidentified specimens. No *C. viridis* were encountered during the survey. A separate collection of \approx 230 specimens including spittlebugs, Deltocephalinae, and Fulgoro-morpha were pinned and preserved for future reference at the Department of Soil, Plant, and Food Science, University of Bari Aldo Moro, Bari, Italy (DiSSPA-UNIBA).

Prevalence of *X. fastidiosa* in Field-Collected Insects. Seventy percent of the *P. spumarius* collected in the first week of November were PCR-positive for *X. fastidiosa*. This decreased to 60% by the third week (Table 1). None of 10 control *P. spumarius* collected from Bari Province were positive for *X. fastidiosa*. All *E. lineolatus* and *P. spumarius* collected in December and January tested negative for *X. fastidiosa* (Table 1). Sequences from *X. fastidiosa*-positive *P. spumarius* showed 100% sequence identity with *X. fastidiosa* from infected field trees. *X. fastidiosa* sequences from *P. spumarius* were deposited in the NCBI database under the accession numbers KJ631115, KJ631116, KJ631117, and KJ631118.

Transmission of *X. fastidiosa* by *P. spumarius*. One month after IAP, two out of the five periwinkle test plants exposed to *P. spumarius* were positive for *X. fastidiosa* by PCR; while no infections were detected in the seven olive test plants after 3 mo. Results were confirmed at 2 and 3 mo after IAP by PCR assays. Sequence analysis of 16S rDNA and *gyrB* gene products from PCR of infected periwinkle plants showed 100% identity with sequences from infected olives. *X. fastidiosa* sequences from periwinkle were deposited in the NCBI database under the accession numbers: KJ406258, KJ406259, and KJ406260. As further proof that *X. fastidiosa* was transmitted to periwinkle, electron micrographs from the PCR-positive PW1 periwinkle plant showed presence of *X. fastidiosa* cells (Fig. 3).

Discussion

X. fastidiosa is a quarantine pathogen in the European Union (Annex I, Part A, Section I to Council Directive 2000/29/EC). Discovery of *X. fastidiosa* in September 2013 in southern Italy prompted an urgent

search for insect vectors of the bacterium. *Xylella fastidiosa* is transmitted by a wide range of xylem-fluid feeding insects (Purcell, 1989, Almeida et al. 2005). Numerous species of xylem fluid-feeding sharpshooters and spittlebugs are known to transmit the bacterium worldwide, but the vector in Italy was not known. To this end, *P. spumarius* collected from the Salento Peninsula were tested by PCR to determine *X. fastidiosa* infectivity and placed on healthy periwinkle and olive plants to determine if they could transmit the pathogen in laboratory tests. The transmission trials showed unambiguously that *P. spumarius* from OQDS-symptomatic olive groves were naturally infectious with *X. fastidiosa* in November and transmitted the bacterium to periwinkle test plants. Additional vector tests with a larger sample size must be performed to prove or disprove *X. fastidiosa* transmission by *P. spumarius* to olives. These results, however, were the first demonstration that the common and widespread insect, *P. spumarius*, can have high rates ($>$ 50%) of infection with *X. fastidiosa* in Italian olive orchards.

Further research is needed to determine seasonal dynamics of vectors harboring *X. fastidiosa* and occurrence of other possible vectors and their ability to transmit *X. fastidiosa* to olive and other hosts. Regardless of the role of *X. fastidiosa*, olive strain, in the etiology of OQDS, results presented in this study on *X. fastidiosa*-inoculative vectors and insect transmission in southwestern Apulia are relevant. This is because of the wide host range of the bacterium and the economic damage it causes to important perennial crops and landscape plants. Moreover, this report identifies a need to develop a regional strategy for the interdiction, control, or management of diseases caused by *X. fastidiosa* and its vectors in Italy as well as in other Mediterranean countries where the pathogen is a quarantine pest.

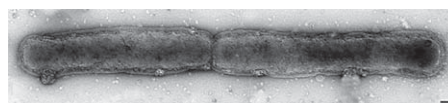


Fig. 3. Electron micrograph of *X. fastidiosa* cells in leaf dip of periwinkle plant showing the bacterium was transmitted by *P. spumarius*. Test insects were collected from ground vegetation in olive orchards symptomatic for OQDS in the Salento Peninsula, Italy. Scale bar, 100 nm.

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