Entomologia Generalis, Vol. 40 (2020), Issue 2, 125–132
Published online 20 February 2020

No evidence for cicadas' implication in *Xylella fastidiosa* epidemiology

Daniele Cornara[1], Monica Marra[2], Biagio Tedone[2], Vincenzo Cavalieri[3], Francesco Porcelli[2], Alberto Fereres[1], Alexander Purcell[4], and Maria Saponari[3]

[1] Instituto de Ciencias Agrarias, Consejo Superior de Investigaciones Cientificas, ICA-CSIC, Calle Serrano 115 dpdo, 28006 Madrid, Spain
[2] DiSSPA, sez, Zoologia ed Entomologia Agraria, Università degli Studi di Bari Aldo Moro, via Amendola 165/A, 70126 Bari, Italy
[3] Istituto per la Protezione Sostenibile delle Piante IPSP, CNR, Via Amendola 122/D, 70126 Bari, Italy
[4] ESPM, University of California Berkeley, 130 Mulford Hall 3114, 94720 Berkeley, CA, USA
* Corresponding author: danielecornara@gmail.com

With 1 figure

Abstract: Cicadas are prominent insects in the Mediterranean basin environments, including in olive orchards. The bacterium *Xylella fastidiosa* is a xylem-limited vector-borne plant pathogen that was introduced into southern Italy, causing significant losses of olive trees. Cicadas are xylem-sap feeders and potential vectors of *X. fastidiosa*; however, knowledge of their role in the transmission of this bacterium is limited. We carried out two separate experiments: the first in California (USA), where we verified the capability of *Platypedia minor* to transmit *X. fastidiosa* from infected to healthy grapevines; the second in Apulia (South Italy), where we evaluated if *Cicada orni* collected on infected olive plants were able to transmit the bacterium to healthy olives. In California, transmission was not achieved for the 19 grapevines caged each with a group of two to three *P. minor*; moreover, none of the 19 insects (out of the 47 used for the transmission test) tested by culturing resulted positive for *X. fastidiosa*. In Italy, none of the olive recipient plants either caged with groups of three *C. orni* individuals per plant confined in sleeve cages (55 plants) or placed inside a mesocosm with cicadas free to move among the recipient plants (30 plants), were infected with the bacterium. Moreover, out of the 314 field collected *C. orni* tested by qPCR, only 4 (1.27%) were positive for the bacterium. Our data suggest that the cicada species we tested likely have no or a negligible role in the natural spread of *X. fastidiosa*.

Keywords: Olive, *Cicada orni*, *Platypedia minor*, Olive Quick Decline Syndrome, grapevine, vector-borne plant pathogens

1 Introduction

Auchenorryncha are a major component of the plant feeding fauna of terrestrial ecosystems on all continents (Bartlett et al. 2018). Furthermore, they are particularly relevant as vectors of plant pathogens (Nault 1997, Backus 1985, Fereres & Moreno 2009). The intensity of a plant pathogen vector can be expressed as a product of its propensity per its activity, thus of the probability the vector inoculate the pathogen multiplied per the number of individuals alighting on the host plant for a certain period of time (Irwin & Ruesink 1986). Therefore, at a theoretical level, the more abundant is the insect vector on the host of the plant pathogen, the greater will be its intensity, thus the greater its epidemiological impact. Considering olive orchards in the Mediterranean, threatened by Olive Quick Decline Syndrome (OQDS) caused by the bacterium *Xylella fastidiosa* that have already devastated olives in Apulia (South Italy) (Saponari et al. 2018), cicadas, such as *Cicada orni* L. (1758) (Hemiptera: Cicadidae), are among the most numerous Hemiptera inhabiting these agro-ecosystems (Patterson 1997, Pinto-Juma et al. 2005). Cicadas are xylem-sap feeders (Cheung & Marshall 1973, Novotny & Wilson 1997). Xylem-sap feeding appears to be the key feature required for being a competent vector of *X. fastidiosa* (Frazier 1965, Almeida et al. 2005); therefore, their feeding behavior make cicadas possible vector of the bacterium. Currently, the spittlebug *Philaenus spumarius* L. (1758) (Hemiptera: Aphrophoridae) is considered the main vector of *X. fastidiosa* to olive in southern Italy, and likely to other host plants in

DOI: 10.1127/entomologia/2020/0912

all the bacterial European outbreaks reported so far (Cornara et al. 2018, 2019, Cruaud et al. 2018, Morente et al. 2018, EFSA 2018, Markheiser et al. 2020). The spittlebug offsets its relatively low vector propensity with a great activity on olive plants that exponentially increases the probability of *X. fastidiosa* transmission (Cornara et al. 2016, 2017a, 2017b). Similarly, the great activity of cicadas on olive plants would make them fearsome vectors in case their vector ability was to be demonstrated. Currently, two reports on cicadas' ability to transmit the bacterium are available, but both provide limited or unavailable data (EFSA 2015). Paião et al. (2002) reported the finding of *X. fastidiosa* in the 35% of the individuals belonging to five cicada species collected in a coffee plantation in Brazil. Moreover, the authors assessed the natural infectivity of *Dorisiana viridis* Olivier (1790) (Hemiptera: Cicadidae) individuals collected in the same field through a transmission test, with 20-30 field collected cicadas caged per each coffee plant. Eventually, transmission occurred on four of the 101 recipient plants used, however full experimental details and datasets are not available. The second report is about a traditional acquisition/inoculation experiment carried out with *Diceroprocta apache* Davis (1921) (Hemiptera: Cicadidae) in California (USA) (Krell et al. 2007). Cicadas collected in a vineyard were given an acquisition access period of 48 hours on four infected grapevine cv Red Globe, then caged singly 48 hours for inoculation on healthy grape plants. *X. fastidiosa* transmission by *D. apache* occurred for one out of 12 recipient plants tested, thus confirming the vector competence of this cicada species. Moreover, a single cicada different from the one that transmitted the bacterium to the recipient plant tested positive for *X. fastidiosa* by PCR. Although the Californian report is more detailed than the Brazilian one, twelve test plants represent a limited dataset. However, it should be remarked that the work by Krell et al. was not just focused on cicadas, but on scarcely explored transmission possibilities, such as pruning shears and root grafts.

The pest risk assessment carried out by the European Food Safety Agency on *X. fastidiosa* highlights high uncertainties about the role of cicadas in spreading *X. fastidiosa*. Given these insects' abundance and distribution in all the Mediterranean areas threatened by *X. fastidiosa* or where the bacterium is already established and relentless spreading, gathering evidences on the role of cicadas as vectors of the bacterium is of high relevance (EFSA 2015).

To this end, we carried out several experiments aimed at verifying the role of cicadas as vectors of *X. fastidiosa*. The first experiment, carried out in California, was focused on the assessment of the acquisition/inoculation ability of *Platypedia minor* Uhler (1888) (Hemiptera: Cicadidae), a cicada species that emerge early in spring, which could play an important role in *X. fastidiosa* primary spread and instauration of chronic infections in grapevines and other crops (Purcell 1981). The second set of experiments consisted of transmission tests using specimens of *C. orni* collected in *X. fastidiosa*-infected olives in southern Apulia.

2 Materials and methods

2.1 Transmission tests with *Platypedia minor*

Platypedia minor individuals, both males and females, were collected by sweep net from olive canopies, grapevines and poplars, in an olive orchard surrounded by vineyards in Petaluma (CA), once a week, from March to May 2015. Immediately after collection, individuals were temporary caged on 2-year old 50 cm tall grapevines (cv. Cabernet Sauvignon), within wood and cloth mesh cages fastened to the tops of 2 L plastic pots, and moved to the lab. Once in the lab, cicadas were given an acquisition access period (AAP) of 48 hours on three 2-year old Thompson seedless cuttings infected with *X. fastidiosa fastidiosa* STL strain, pooling the entire group of individuals collected in the field onto the source plants within a methacrylate and mesh cage (1×1×1 m). The source plants had been infected with *X. fastidiosa* by pin-prick the year before our experiment, following the method described by Almeida & Purcell (2003). For the experiment, we selected source plants with a *X. fastidiosa* population of around 10^7CFU/g of tissue, as determined by culturing and dilution plating (Hill & Purcell 1995). After the AAP, during which cicadas were observed (at least once) alighting on the grape and staying over the tissues with stylets inserted, alive individuals were transferred until they died to recipient 2-year old Cabernet Sauvignon rooted cuttings, in groups of two or three insects per plant, within wood and mesh cages. Eventually, a total of 19 grapevines were caged with *P. minor* individuals upon the AAP, of which ten and nine plants caged with two and three cicadas each, respectively. The inoculation access period (IAP), corresponding to the time the cicadas survived on the recipient plants, ranged approximately from 96 to 240 hours. At the end of the IAP, a part of the cicadas was pinned and stored as reference collection. Fourteen out of the 47 cicadas caged for the IAP were tested for *X. fastidiosa* by culturing, according to Hill & Purcell (1995). Briefly, suddenly after the end of the IAP, the entire cicada body was sterilized through successive immersion in 90% ethanol, 2% hypochlorite, and three rinses in sterile distilled water. Thereafter, head, thorax and abdomen were severed, and each part was firstly chopped with a sterile razor blade, then ground by pestle in 1 ml succinate-phosphate buffer inside a mortar. Finally, two 20µl drops of the extract were plated on PWG substrate; plates were incubated upside-down for seven days at 28°C. Recipient plants were stored in a greenhouse, with temperature of 25.6±6.4°C, watered daily, fertilized monthly (Osmocote Plus 15-9-12), and treated with insecticides biweekly as necessary. Eventually, one, three and five months after IAP, both the 19 recipient plants and the nine plants on which the cicadas

were caged during the transfer from the field to the lab, were tested for *X. fastidiosa* by culturing, following the method described by Hill & Purcell (1995).

2.2 Natural infectivity of *Cicada orni*

Two transmission tests onto olive recipient plants using *C. orni* individuals collected from infected olives were conducted. The first test was carried out by using a protocol commonly used in traditional transmission experiments, collecting cicadas in the field and caging them on recipient plants in groups of three insects per plant in sleeve cages. For the second test, upon collection, all the cicadas were released inside an insect rearing mesocosm in which potted olive recipient plants were placed. Thus, with insects free to fly, move among the plants, and select the favorite portions to alight and feed, without being constrained in a limited space, we attempted to eliminate the possible negative effect of small confined space on the insects' behavior.

For the first test, *C. orni* males and females were collected during 2016 in an olive orchard in the municipality of Salve (Lecce, South Italy) with an estimated incidence of *X. fastidiosa* infections higher than 70% as determined by qPCR (olive plants varieties Ogliarola leccese and Cellina di Nardò). Cicadas were captured on olive canopies by sweep net, daily, during the last week of July and the first week of August. Once collected, cicadas were transferred into an empty wood and mesh cage of 1×1×1 m, and fasted for 2 hours. Thereafter, *C. orni* individuals were caged with sleeve net cages in groups of three per plant on 55 olive plants (1.5m in height) until death. The sleeve cages were put in place in order to fit the entire recipient plants until the base (excluding the pot), leaving to the insect the space to move. Twenty plants caged without insects during the IAP were used as negative control. Additionally, as positive control, five healthy olive plants were caged each with a group of five *P. spumarius* collected in the same olive orchard where the cicadas were collected, for an IAP of 96 hours. Transmission experiments were carried out in a screen-house located in Parabita (Lecce, South Italy), at temperature 30.3 ± 5.8°C. At the end of the IAP, the cicadas were stored in EOTH 70% at −20°C until the qPCR.

For the second experiment, carried out during summer 2018, the cicadas were collected once a week in the same field selected in 2016. Approximately 45 cicadas per week were collected on olive canopies by sweep net for five consecutive weeks during the period June-July (a total of 234 individuals collected). An insect rearing mesocosm (height: 2m; length: 4m; width: 2m) covered with a shadowing net was installed at the center of an empty field of approximately 1ha located in Racale (Lecce, South Italy). Ground vegetation was removed by manual tillage from an area approximately nine times the extension of the mesocosm before placing the mesocosm itself. Thirty recipient olive plants were arranged inside the tent on six rows, with five plants per row. Two myrtle leaf milkwort (*Polygala myrtifolia*) plants were placed one inside and one outside the mesocosm and used as sentinel plants. Once a week upon the collection, the cicadas were released inside the mesocosm; therefore, the olive plants were continuously exposed to groups of cicadas collected in the *X. fastidiosa* infected olive orchard for five consecutive weeks, with each cicada given an IAP corresponding to its survival time inside the mesocosm. Temperature and humidity inside the mesocosm were measured with a data-logger; the average daily temperature and humidity during the IAP ranged ca. from 23 to 30°C and from 55 to 80%, respectively. Twice per day (approximately at 10 am and 5 pm) the tent was accurately screened (first from outside then from the inside) in order to observe if cicadas were alighting on and probing the olive recipient plants, and collect the dead insects. Dead cicadas that could be spotted inside the cage were stored in EOTH 70% at −20°C until the qPCR.

The olive plants used as recipient plants either in 2016 and 2018 were five-year old olives var. Cellina di Nardò reared in a non-conditioned insect-proof screenhouse at CRSFA Basile Caramia (Locorotondo, Bari, South Italy). These plants were tested by qPCR for *X. fastidiosa* (following the protocol by Loconsole et al. (2014)) before starting the experiments, confirming they were negative. At the end of the IAP, the recipient plants were treated with a broad spectrum insecticide (Decis Evo, a.i. Deltamethrin, Bayer, 50ml/hl), then moved to an insect-free screenhouse in Gallipoli (Lecce, South Italy), watered once a week, and treated with the same insecticide every three weeks. Nine and 12 months after the IAP, the olives (and the myrtle leaf milkwort used as control in 2018) were tested for the bacterium by qPCR following Loconsole et al. (2014). Diagnostic tests on cicadas were performed on the excised heads by qPCR (Harper et al. 2010) upon extracting the DNA using a CTAB-based procedure (EPPO 7/24 3).

3 Results

3.1 Transmission tests with *Platypedia minor*

For all the groups of cicadas, we observed at least one individual per group probing the host plants multiple times and for several minutes, either the recipient plants or the ones used for the transfer to the lab (usually cicadas were observed on the main stem of the plant). Both the recipient plants (19) and the plants on which cicadas were placed upon the collection for the transfer to the lab (9), tested negative for the bacterium. Similarly, the fourteen cicadas tested by culturing were negative for *X. fastidiosa*.

3.2 Natural infectivity of *Cicada orni*

In the 2016 trial, we observed the cicadas probing for several minutes either shoots or trunks of 15 out of 55 recipient

plants. The IAP ranged from 24 (14 out of 55 cases) to 120 hours (2 out of 55). The mean survival time, thus the mean IAP was 51.9±23.9 hours. Three out of 165 cicadas tested positive for *X. fastidiosa* by qPCR; one positive individual belonged to a group whose individuals were observed probing the recipient plant. Two of the three positive *C. orni* belonged to groups of individuals that survived just 24 hours on the recipient plant, whereas the other one likely had an IAP of 48 hours. Both olives caged with field collected cicadas and negative controls tested negative for *X. fastidiosa*. On the contrary, three out of five plants caged with naturally infective *P. spumarius* were positive for *X. fastidiosa*.

Regarding the experiment conducted in 2018, a total of 149 cicadas (out of the 234 collected and introduced in the cage with the healthy olive plants) were recovered and tested by qPCR; the remaining 85 cicadas were either discarded because desiccated and with detached heads (51), or because lost during the experiment (34). All the olive plants inside the mesocosm were visited at least once by the insects that were observed probing either the trunks or the lateral shoots. Only one cicada out of the 149 tested was found positive to *X. fastidiosa* by qPCR. None of the olive recipient plants tested positive to *X. fastidiosa*, neither nine nor 12 months after the IAP. Additionally, the myrtle leaf milkworts, used as control, were negative too for the fastidious bacterium.

4 Discussion

None of the transmission experiments carried out with the two cicada species tested resulted in successful transmission of *X. fastidiosa* to the recipient grapevine and olive plants. Regarding transmission experiment with *P. minor* on grape, our results could have been influenced by the cicadas' feeding behavior in captivity, and/or limited dataset. Considering the feeding behavior, although in field in California we found cicadas on grapevines, we collected *P. minor* mainly on olive plants, suggesting a preference for this host plant. Nevertheless, although tissue probing does not necessarily involve feeding, we observed the cicadas inserting the stylets and remaining on the probed tissues, either on sources or on the recipient grapevines. Feeding behavior and vector host range are likely the key factors in *X. fastidiosa* transmission (Almeida et al. 2005; Daugherty et al. 2010). Thus, transmission gathered by Krell et al. (2007) with *D. apache* may indicate the preference of this species for grapevine. It should be also remarked that even if in the report of Sanborn & Phillips (2013) grapevine is not specifically listed among the host plants of *D. apache*, the widespread occurrence of these cicada species in riparian habitats, where *Vitis* spp. frequently occur, may be an indirect evidence of a preference for this host. Regarding the limited dataset, with 19 plants inoculated with more than one cicada for a time ranging from 96 to 240 hours (28 considering the nine grapevines used for the transfer to the lab), compared to single-individual 48 hour transmission test performed by Krell et al. (2007), we used both a greater number of individuals per recipient plant, and a longer IAP. This, according to Daugherty & Almeida (2009), should have exponentially increased the transmission probability. In addition, discrepancy between Krell's results and ours could be even associated to morphological or physiological differences of vector species belonging to different subfamilies (*P. minor* belongs to subfamily Tibicininae, *D. apache* to subfamily Cicadinae) which may influence *X. fastidiosa* transmission dynamics. Eventually, more transmission studies with several cicada species and host plants should be performed in order to definitely assess the likelihood of cicadas involvement in *X. fastidiosa* transmission in California. Whereas the epidemiological role of cicadas in California seems to be negligible compared to sharpshooters, in Europe the scenario seems to be the reverse; sharpshooters are scantily present, and spittlebugs and cicadas shall likely be the most important candidate vectors (Cornara et al. 2018, Cornara et al. 2019). While first data on spittlebug's role in *X. fastidiosa* transmission in different European outbreaks have been reported (Cornara et al. 2017a, b, Cruaud et al. 2018, EFSA 2018, Cavalieri et al. 2019), nothing is known yet about the relationship between *X. fastidiosa* and the cicadas (EFSA 2015). *Cicada orni* is a very common and abundant species within olive orchards of Salento (Cornara, pers. obs.), the southernmost region of Apulia (Italy), where the bacterium *X. fastidiosa*, the causal agent of OQDS, was first detected and is currently established and spreading (Saponari et al. 2018). Cicadas are among the largest and most numerous insects in the habitat where they occur (Patterson et al. 1997, Simoes & Quartau 2007). Their large size, frequent flights, and loud courtship calls make them very noticeable in olive orchards. Furthermore, they are important both as component of the ecosystem (Andersen 1987, 1994) and as prey (Rosenberg 1982, Ciampalini & Lovari 1985, Pigozzi 1991). Given these premises, the assessment of the natural infectivity of cicadas, and their possible role in *X. fastidiosa* natural spread, are issues of high relevance for the entire Mediterranean area and the countries threatened by the fastidious bacterium (EFSA 2015).

Following the qPCR protocol by Harper et al. (2010), we found three *C. orni* positive to *X. fastidiosa* out of 165 sampled (1.8%) in 2016, and one out of 149 in 2018 (0.67%). Overall, considering the two years of collection, four out of 314 cicadas, thus ca. 1.27% of the individuals, were found bearing the bacterium. This value is lower than that reported by Krell et al. (2007) for *D. apache* (8.3%), and the ones reported by Paião et al. (2002) for the five species tested (ca. 35%).

Moreover, no transmission of *X. fastidiosa* to olive recipient plants occurred. According to Bextine et al. (2004), insect body contains many potential DNA-inhibitors, especially pigments present in insect's eyes. Thus, one may hypothesize that detection of *X. fastidiosa* cells through PCR in large cicada bodies could be particularly challeng-

ing. Additionally, bacterium detection in an insect through molecular methods cannot prove or disprove the species role as bacterium vector (EFSA 2015). Indeed, insects hosting a *X. fastidiosa* population lower than detection threshold can efficiently transmit the bacterium (Hill & Purcell 1995, Cornara et al. 2016). Secondly, species that usually feed on tissues other than xylem vessels, may probe the xylem (Pompon et al. 2011), possibly acquiring *X. fastidiosa* cells without being capable of transmitting the bacterium (Purcell 1980). The last one is not the case of cicadas, reported as xylem-feeder (Cheung & Marshall 1973); nevertheless, the precise feeding behavior of these very large xylem-feeders is unknown, and it is possible that their large stylets could disrupt the tissue interrupting the steady flow necessary for *X. fastidiosa* cells to start plant tissues colonization (Purcell et al. 1994). It is also possible that the very low acquisition rate and the lack of inoculation could be the result of the limited life-span of *C. orni*, not longer than 1–2 weeks (Simões & Quartau 2007), during which cicadas possibly perform few probes. This would lead to reduced possibilities to encounter a xylem vessel containing the bacterium for acquisition, and a likely null or extremely low probability of inoculation (Jackson et al. 2008, Daugherty & Almeida 2009). Additionally, lack of transmission could also be related with a dynamic of fluids within *C. orni* foregut different from that reported for ascertained vectors as *P. spumarius* (Cornara et al. 2018, Ruschioni et al. 2019) that could potentially hinder bacterial cells retention and successive inoculation.

For the experiment performed in 2016, the lack of transmission could also have been the outcome of cicadas' manipulation during a traditional transmission experiment. Both in the Californian and in the Italian experiment, we observed a high mortality rate of caged cicadas. In 2016, the 25.45% of *C. orni* (14 out of 55) died within 24 hours from the caging, and the mean survival was of ca. two days, even on a well-known host plant for *C. orni* as olive. Irwin & Ruesink (1986) stated that aphid feeding behavior is strongly affected by caging, thus for estimating vector ability under field conditions (vector propensity), insects need to be free to fly and move from plant to plant and feed in a natural way under free choice conditions. For *X. fastidiosa* no vector-strain specificity occurs, and feeding behavior seems to be the key feature shared by the vectors of the bacterium (Almeida et al. 2005). Therefore, if cicadas feeding behavior is strongly affected by caging, classical transmission tests should not furnish a real picture about their epidemiological role in *X. fastidiosa* transmission. This could drive to an underestimation of the role of a vector, especially for vectors with a transmission efficiency as low as 1%. Because of the widespread abundance of cicadas on Mediterranean olive trees, even a small rate (e.g., below 1%) of transmission of *X. fastidiosa* could account for serious rates of spread of OQDS. The impact of very low rates of transmission is especially relevant for large, long-lived plants such as olive trees (Purcell 1980). By carrying out a power analysis for data with a binomial distribution, we calculated that, in order to achieve a statistically significant result (95% confidence level) with a transmission efficiency of 1%, sample size should be 154 plants for a statistical power of 80%, and 67 plants for a statistical power of 50% (Fig. 1). It has to be said that, whereas sample size has been calculated considering one single insect for each recipient plant, for our experiments in 2016 we caged more

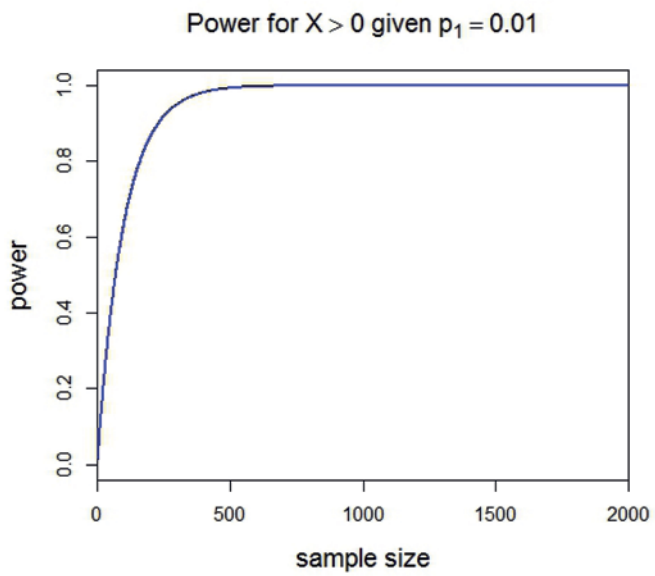

Fig. 1. Power analysis for binomial distribution (transmission efficiency 1% or lower).

than one cicada per plant, thus increasing the transmission probability (Daugherty & Almeida 2009). Eventually, from a statistical point of view, the failure in transmission with *C. orni* to olive in 2016 cannot be attributed to inadequate sample size, but could be the by-product of cicadas' manipulation. However, the results of the tests carried out in 2018, when we used a mesocosm and cicada individuals were free to fly and move among the recipient plants, thus with far less behavioral constrains compared to 2016, likely rule out the possibility that lack of transmission is related to manipulation and caging for the transmission test. Furthermore, even if *C. orni* was able to transmit the bacterium (with a very reduced efficiency hard to demonstrate under experimental conditions) its contribution to OQDS epidemiology would be negligible compared to the meadow spittlebug *P. spumarius*, considering the life span of the two insects. Indeed, adult spittlebugs live several months after acquisition of the bacterium from infected olives, moving toward olive plants twice a year and spending on this host plant a relatively long time interval (first from sprouting to flowering/fruits setting, then in autumn when the insect is attracted by olive suckers) (Cornara et al. 2017b). Therefore, the olives are exposed twice a year and for several weeks/months to abundant potentially infective spittlebug populations, thus to a great vector load prolonged in time. On the contrary, even if abundant on olive, *C. orni* adults have a mean life-span of one week (Simões & Quartau 2007); thus, the vector load would be great but limited in time, and overall negligible if compared to *P. spumarius*.

Eventually, our data suggest that: i) *P. minor* does not have, or have a very negligible role, in *X. fastidiosa* transmission to grapevine in California; ii) *C. orni* is likely not involved in *X. fastidiosa* transmission from olive to olive in the epidemics occurring in southern Apulia. Overall, it appears likely that cicadas, even if occasionally capable of acquiring *X. fastidiosa*, have likely a very small, perhaps negligible, role in the natural spread of the bacterium. However, further tests with cicada-host plant systems different from the ones we tested here are needed in order to gather more evidences about cicadas' competence in transmitting *X. fastidiosa*.

Our conclusions raise further questions about cicadas feeding behavior and the reasons underlying the failure (at least by the two species tested) of transmitting *X. fastidiosa* by insects considered as xylem-feeders. The answers to such challenging questions might furnish new important insights into the relationship vector-*X. fastidiosa*, opening new venues toward the environmentally sustainable control of the fastidious bacterium through the disruption of the transmission process.

Author Contribution: DC and FP conceived research. DC, BT, MM, VC and AP conducted experiments. DC analyzed the data. DC wrote the manuscript. FP, AF, AP and MS reviewed and edited the manuscript. FP, AF, AP and MS secured funding. All authors read and approved the manuscript.

Data availability statement: Additional data will be furnished by the authors upon reasonable request.

Competing interests statement: The authors declare no competing interests.

Acknowledgements: We are deeply thankful to Enzo Manni and Federico Manni (Coop. ACLI-Racale) for the use of the rearing and transmission facilities. We acknowledge Francesco Palmisano, Crescenza Dongiovanni, and Giulio Fumarola (CRSFA-Basile Caramia) for plants rearing, and Nicola Bodino (IPSP-CNR Turin) and Nicola Tagarelli (DiSSPA, University of Bari) for support in field activities. We also acknowledge Giuseppe Altamura and Raied Abou Kubaa (IPSP-CNR Bari) for technical support in laboratory analysis. This work has been financially supported by European Union Horizon 2020 research and innovation program under grant agreements no. 635646 POnTE (Pest Organisms Threatening Europe).

References

Almeida, R. P., & Purcell, A. H. (2003). Biological traits of *Xylella fastidiosa* strains from grapes and almonds. *Applied and Environmental Microbiology, 69*(12), 7447–7452. https://doi.org/10.1128/AEM.69.12.7447-7452.2003

Almeida, R. P. P., Wistrom, C., Hill, B. L., Hashim, J., & Purcell, A. H. (2005). Vector transmission of *Xylella fastidiosa* to dormant grape. *Plant Disease, 89*(4), 419–424. https://doi.org/10.1094/PD-89-0419

Andersen, D. C. (1987). Below-ground herbivory in natural communities: A review emphasizing fossorial animals. *The Quarterly Review of Biology, 62*(3), 261–286. https://doi.org/10.1086/415512

Andersen, D. C. (1994). Are cicadas (*Diceroprocta apache*) both a "keystone" and a "critical-link" species in lower Colorado River riparian communities? *The Southwestern Naturalist, 39*(1), 26–33. https://doi.org/10.2307/3672188

Backus, E. A. (1985). Anatomical and sensory mechanisms of planthopper and leafhopper feeding behavior. In L. R. Nault & J. G. Rodriguez (Eds.), *The leafhoppers and planthoppers* (Vol. 10, pp. 163–194). New York, N.Y: John Wiley & Sons, Inc.

Bartlett, C. R., Deitz, L. L., Dmitriev, D. A., Sanborn, A. F., Soulier-Perkins, A., & Wallace, M. S. (2018). The Diversity of the True Hoppers (Hemiptera: Auchenorrhyncha). In R. G. Foottit & P. H. Adler (Eds.), *Insect Biodiversity: Science and Society* (Vol. II, pp. 501–590). Chichester, UK: Wiley Blackwell; https://doi.org/10.1002/9781118945582.ch19

Bextine, B., Tuan, S.-J., Shaikh, H., Blua, M., & Miller, T. A. (2004). Evaluation of methods for extracting *Xylella fastidiosa* DNA from the glassy-winged sharpshooter. *Journal of Economic Entomology, 97*(3), 757–763. https://doi.org/10.1603/0022-0493(2004)097[0757:EOMFEX]2.0.CO;2

Cavalieri, V., Altamura, G., Fumarola, G., di Carolo, M., Saponari, M., Cornara, D., … Dongiovanni, C. (2019). Transmission of

Xylella fastidiosa subspecies *pauca* Sequence Type 53 by different insect species. *Insects, 10*(10), 324. https://doi.org/10.3390/insects10100324

Cheung, W. W. K., & Marshall, A. T. (1973). Water and ion regulation in cicadas in relation to xylem feeding. *Journal of Insect Physiology, 19*(9), 1801–1816. https://doi.org/10.1016/0022-1910(73)90049-8

Ciampalini, B. (1985). Food habits and trophic niche overlap of the badger (*Meles meles* L.) and the red fox (*Vulpes vulpes* L.) in a Mediterranean coastal area. *Zeitschrift fur Saugetierkunde, 50*, 226–234.

Cornara, D., Bosco, D., & Fereres, A. (2018). *Philaenus spumarius*: When an old acquaintance becomes a new threat to European agriculture. *Journal of Pest Science, 91*(3), 957–972. https://doi.org/10.1007/s10340-018-0966-0

Cornara, D., Cavalieri, V., Dongiovanni, C., Altamura, G., Palmisano, F., Bosco, D., … Saponari, M. (2017a). Transmission of *Xylella fastidiosa* by naturally infected *Philaenus spumarius* (Hemiptera, Aphrophoridae) to different host plants. *Journal of Applied Entomology, 141*(1–2), 80–87. https://doi.org/10.1111/jen.12365

Cornara, D., Morente, M., Markheiser, A., Bodino, N., Tsai, C. W., Fereres, A., … Lopes, J. R. S. (2019). An overview on the worldwide vectors of *Xylella fastidiosa. Entomologia Generalis, 39*(3-4), 157–181. https://doi.org/10.1127/entomologia/2019/0811

Cornara, D., Saponari, M., Zeilinger, A. R., de Stradis, A., Boscia, D., Loconsole, G., … Porcelli, F. (2017b). Spittlebugs as vectors of *Xylella fastidiosa* in olive orchards in Italy. *Journal of Pest Science, 90*(2), 521–530. https://doi.org/10.1007/s10340-016-0793-0

Cornara, D., Sicard, A., Zeilinger, A. R., Porcelli, F., Purcell, A. H., & Almeida, R. P. P. (2016). Transmission of *Xylella fastidiosa* to grapevine by the meadow spittlebug. *Phytopathology, 106*(11), 1285–1290. https://doi.org/10.1094/PHYTO-05-16-0202-R

Cruaud, A., Gonzalez, A.-A., Godefroid, M., Nidelet, S., Streito, J.-C., Thuillier, J.-M., … Rasplus, J.-Y. (2018). Using insects to detect, monitor and predict the distribution of *Xylella fastidiosa*: A case study in Corsica. *Scientific Reports, 8*(1), 15628. https://doi.org/10.1038/s41598-018-33957-z

Daugherty, M. P., & Almeida, R. P. P. (2009). Estimating *Xylella fastidiosa* transmission parameters: Decoupling sharpshooter number and feeding period. *Entomologia Experimentalis et Applicata, 132*(1), 84–92. https://doi.org/10.1111/j.1570-7458.2009.00868.x

Daugherty, M. P., Lopes, J., & Almeida, R. P. (2010). Vector within-host feeding preference mediates transmission of a heterogeneously distributed pathogen. *Ecological Entomology, 35*(3), 360–366. https://doi.org/10.1111/j.1365-2311.2010.01189.x

EFSA. (2015). Scientific Opinion on the risk to plant health posed by *Xylella fastidiosa* in the EU territory, with the identification and evaluation of risk reduction options. *EFSA Journal, 13*(1), 3989. https://doi.org/10.2903/j.efsa.2015.3989

EFSA. (2018). Updated pest categorisation of *Xylella fastidiosa. EFSA Journal, 16*(7).

EPPO. (2018). PM 7/24-3 *Xylella fastidiosa. Bulletin OEPP. EPPO Bulletin. European and Mediterranean Plant Protection Organisation, 48*, 175–218. https://doi.org/10.1111/epp.12469

Fereres, A., & Moreno, A. (2009). Behavioural aspects influencing plant virus transmission by homopteran insects. *Virus Research, 141*(2), 158–168. https://doi.org/10.1016/j.virusres.2008.10.020

Frazier, N. W. (1965). Xylem viruses and their insect vectors. In: Proceedings of the international conference on virus and vectors on perennial hosts, with special reference to *Vitis* 91–99.

Harper, S. J., Ward, L. I., & Clover, G. R. G. (2010). Development of LAMP and real-time PCR methods for the rapid detection of *Xylella fastidiosa* for quarantine and field applications. *Phytopathology, 100*(12), 1282–1288. https://doi.org/10.1094/PHYTO-06-10-0168

Hill, B. L., & Purcell, A. H. (1995). Acquisition and retention of *Xylella fastidiosa* by an efficient vector, *Graphocephala atropunctata. Phytopathology, 85*(2), 209–212. https://doi.org/10.1094/Phyto-85-209

Irwin, M. E., & Ruesink, W. G. (1986). Vector intensity: A product of propensity and activity. In R. G. Garrett & W. G. Ruesink (Eds.), *Plant Virus Epidemics: Monitoring, Modelling and Predicting Outbreaks. McLean GD* (Vol. 23, pp. 13–33). Sydney, Australia: Academic Press.

Jackson, B. C., Blua, M. J., & Bextine, B. (2008). Impact of duration versus frequency of probing by *Homalodisca vitripennis* (Hemiptera: Cicadellidae) on inoculation of *Xylella fastidiosa. Journal of Economic Entomology, 101*(4), 1122–1126. https://doi.org/10.1603/0022-0493(2008)101[1122:IODVFO]2.0.CO;2

Krell, R. K., Boyd, E. A., Nay, J. E., Park, Y. L., & Perring, T. M. (2007). Mechanical and insect transmission of *Xylella fastidiosa* to *Vitis vinifera. American Journal of Enology and Viticulture, 58*(2), 211–216.

Loconsole, G., Potere, O., Boscia, D., Altamura, G., Djelouah, K., Elbeaino, T., …. (2014). Detection of *Xylella fastidiosa* in olive trees by molecular and serological methods. *Journal of Plant Pathology, 96*(1), 7–14.

Markheiser, A., Cornara, D., Fereres, A., & Maixner, M. (2020). Analysis of vector behavior as a tool to predict *Xylella fastidiosa* patterns of spread. *Entomologia Generalis*. https://doi.org/10.1127/entomologia/2019/0841

Morente, M., Cornara, D., Plaza, M., Durán, J., Capiscol, C., Trillo, R., … Fereres, A. (2018). Distribution and Relative Abundance of Insect Vectors of *Xylella fastidiosa* in Olive Groves of the Iberian Peninsula. *Insects, 9*(4), 175. https://doi.org/10.3390/insects9040175

Nault, L. R. (1997). Arthropod transmission of plant viruses: A new synthesis. *Annals of the Entomological Society of America, 90*(5), 521–541. https://doi.org/10.1093/aesa/90.5.521

Novotny, V., & Wilson, M. R. (1997). Why are there no small species among xylem-sucking insects? *Evolutionary Ecology, 11*(4), 419–437. https://doi.org/10.1023/A:1018432807165

Paião, F. G., Meneguim, A. M., Casagrande, E. C., & Leite, R. P. (2002). Envolvimento de cigarras (Homoptera, Cicadidae) na transmissão de *Xylella fastidiosa* em cafeeiro. *Fitopatologia Brasileira, 27*, 67.

Patterson, I. J., Massei, G., & Genov, P. (1997). The density of cicadas *Cicada orni* in Mediterranean coastal habitats. *The Italian Journal of Zoology, 64*(2), 141–146. https://doi.org/10.1080/11250009709356187

Pigozzi, G. (1991). The diet of the European badger in a Mediterranean coastal area. *Acta Theriologica, 36*(3–4), 293–306. https://doi.org/10.4098/AT.arch.91-31

Pinto-Juma, G., Simões, P. C., Seabra, S. G., & Quartau, J. A. (2005). Calling song structure and geographic variation in *Cicada orni* Linnaeus (Hemiptera: Cicadidae). *Zoological Studies (Taipei, Taiwan), 44*(1), 81–94.

Pompon, J., Quiring, D., Goyer, C., Giordanengo, P., & Pelletier, Y. (2011). A phloem-sap feeder mixes phloem and xylem sap to regulate osmotic potential. *Journal of Insect Physiology, 57*(9), 1317–1322. https://doi.org/10.1016/j.jinsphys.2011.06.007

Purcell, A. H. (1980). Almond leaf scorch: Leafhopper and spittlebug vectors. *Journal of Economic Entomology, 73*(6), 834–838. https://doi.org/10.1093/jee/73.6.834

Purcell, A. H. (1981). Vector preference and inoculation efficiency as components of resistance to Pierce's disease in European grape cultivars. *Phytopathology, 71*(4), 429–435. https://doi.org/10.1094/Phyto-71-429

Purcell, A. H., Gravena, S., & Donadio, L. C. (1994). Sharpshooter in citrus crops. Citrus-integrated management of insect and mite pests. Bebedouro, Estação Experimental de Citricultura 213–229.

Rosenberg, K. V., Ohmart, R. D., & Anderson, B. W. (1982). Community organization of riparian breeding birds: Response to an annual resource peak. *The Auk, 99*(2), 260–274.

Ruschioni, S., Ranieri, E., Riolo, P., Romani, R., Almeida, R. P., & Isidoro, N. (2019). Functional anatomy of the precibarial valve in *Philaenus spumarius* (L.). *PLoS One, 14*(2), e0213318. https://doi.org/10.1371/journal.pone.0213318

Sanborn, A. F., & Phillips, P. K. (2013). Biogeography of the Cicadas (Hemiptera: Cicadidae) of North America, North of Mexico. *Diversity (Basel), 5*(2), 166–239. https://doi.org/10.3390/d5020166

Saponari, M., Giampetruzzi, A., Loconsole, G., Boscia, D., & Saldarelli, P. (2018). *Xylella fastidiosa* in olive in Apulia: Where we stand. *Phytopathology, 109*(2), 175–186. https://doi.org/10.1094/PHYTO-08-18-0319-FI

Simões, P. C., & Quartau, J. A. (2007). On the dispersal of males of *Cicada orni* in Portugal (Hemiptera: Cicadidae). *Entomologia Generalis, 30*(3), 245–252. https://doi.org/10.1127/entom.gen/30/2007/245

Manuscript received: 12 August 2019
Revisions requested: 01 October 2019
Modified version received: 18 October 2019
Accepted: 09 January 2020