




Probing behavior of *Neophilaenus campestris* on various plant species

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Abstract

The recent history of *Xylella fastidiosa* Wells et al. introduction in Europe illustrates how the lack of knowledge about the bacterium-vector-host interactions hinders the application of effective containment strategies, with the bacterial spread that currently appears relentless. Vector behavior is a key component in plant pathogen transmission; therefore, detailed knowledge of vector probing behavior on various host plants would furnish useful data for containing the bacterium. Aiming at this goal, we carried out electrical penetration graph (EPG)-assisted probing behavior observations of the spittlebug, *Neophilaenus campestris* (Fallen) (Hemiptera: Aphrophoridae), a candidate vector widespread in all the areas where *X. fastidiosa* is currently established. Spittlebug probing was first characterized on one of its preferred host plants, *Bromus madritensis* L. (Poaceae), over short (6 h) and long (16 h) time spans. Thereafter, we performed comparative observations of the spittlebug probing on *B. madritensis*, olive, and grapevine. Overall, the probing behavior of *N. campestris*, that is, the main characteristic waveforms, did not differ substantially from that of *Philaenus spumarius* (L.) (Hemiptera: Aphrophoridae); however, here we report and describe a new kind of xylem activity interruption, provisionally termed N2, not previously observed in *P. spumarius*. Considering the time spent in xylem ingestion as a percentage of the total probing time (host-suitability indicator used for spittlebugs), grapevine and *B. madritensis* are the preferred hosts for *N. campestris*, with olive as the least suitable among the plants tested. Successful probes, that is, probes during which xylem ingestion occurred, on grapevine were 2.5× greater than olive. Therefore, our data on *N. campestris* probing behavior suggest that the spittlebug role in *X. fastidiosa* epidemiology in European vineyards should be given more attention. In the present manuscript, we also discuss the current difficulties in interpreting and analyzing the outcomes of EPG studies on spittlebugs.

Introduction

Incursion preparedness and pro-active research represent the only valid tools in order to limit the economic losses

associated with the introduction and spread of pests and pathogens (Rathé et al., 2012; Hulme, 2014). This general assumption has been confirmed by events associated with the movement of the plant pathogenic bacterium *Xylella fastidiosa* Wells et al. to Europe: the lack of knowledge about the pathosystem – that is, insect vectors and their interaction with the host plants and the pathogen they transmit – hindered the application of effective containment strategies, with the bacterial spread that currently

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appears relentless. The bulk of literature based on *X. fastidiosa* is in context of the Pierce's disease (PD) pathosystem in California, USA, where sharpshooters play the key role in bacterium epidemiology (Rapicavoli et al., 2018; Cornara et al., 2019). Presumably, an implicated vector in any region is capable of transmitting any strain of *X. fastidiosa*, whether it is endemic or invasive, although with a certain degree of specificity (Almeida & Nunney, 2015; Esteves et al., 2019). Any xylem feeder should be considered a potential vector, but the distribution of xylem feeders varies among countries and regions, and therefore each pathosystem and region might have a predominant vector species (Cornara et al., 2018a). For example, the meadow spittlebug, *Philaenus spumarius* (L.) (Hemiptera: Aphrophoridae), is considered the main vector of the bacterium to olives in southern Italy, where it causes a disease known as Olive Quick Decline Syndrome (OQDS), whereas its role in PD spread is considered marginal in Californian vineyards (Almeida et al., 2005; Cornara et al., 2016). At the same time, another spittlebug, that is, *Neophilaenus campestris* (Fallen) (Hemiptera: Aphrophoridae), whose impact in OQDS is very limited, could play an important role in pathosystems other than olive groves in southern Italy (Cornara et al., 2017). Indeed, abundant populations of *N. campestris* were reported on *Bromus* sp. and *Avena* sp. and other Gramineae in vineyards and olive, almond, and stone-fruit orchards in the Spanish regions of Madrid, Castellon, Alicante, and Balears (Miranda et al., 2017; Morente et al., 2018a, b). In addition, *N. campestris* has been collected on grapevine canopies during independent surveys in Italy, Germany, and northeastern Spain (Arrarás-Oroz et al., 2018; Cornara et al., 2019; A Markheiser, unpubl.; D Cornara, pers. obs.; G Santoemma, pers. comm.).

A key component in vector-borne pathogens is vector behavior, encompassing dispersal ability, host preferences, and alighting and feeding behavior (Irwin & Ruesink, 1986; Fereres & Moreno, 2009; Daugherty et al., 2010). Detailed knowledge of vector probing behavior on various host plants would furnish useful data on host plant range, possible disease epidemiology, and vector control strategies. Here, following the experimental approach illustrated by other authors (Sandanyaka et al., 2013, 2017; Markheiser et al., 2020), we used the electrical penetration graph (EPG) technique (McLean & Kinsey, 1964; Tjallingii, 1978; Backus & Bennett, 2009) to: (1) describe *N. campestris* probing behavior over short and long time periods on one of its favorite host plants, *Bromus madritensis* L. (Poaceae), and (2) gather data on the spittlebug feeding behavioral performances on grapevine and olive in comparison to *B. madritensis*. Overall, the main goal of the present work is to broaden knowledge on a pivotal aspect of

X. fastidiosa epidemiology in Europe, that is, the probing behavior of spittlebugs, the most relevant vector taxon throughout the continent.

Materials and methods

Insects and plants

Neophilaenus campestris nymphs were collected in Morata de Tajuna (Madrid, Spain) in April 2018 on 3-week-old *B. madritensis* plants (further referred to as bromus; seeds were kindly provided by Cesar Fernandez-Quintanilla from the Department of Plant Protection, ICA-CSIC Madrid) grown in 3-l pots filled with a mixture of soil: vermiculite (6:1). Nymphs were transferred with a paintbrush to bromus plants in groups of ca. 20 per plant, caged with a plastic and mesh cylinder, and reared at L16(23 °C):D8 (18 °C) photothermoperiod. The emerging adults were kept under the same conditions described for nymphs, replacing the host plants fortnightly.

Characterization of probing behavior on bromus

Males and females' probing behavior on 3-week-old bromus plants was recorded by EPG, for 6 h (nine records with males, 11 with females). In addition, in order to check for possible diurnal variation (e.g., circadian rhythms) in the spittlebug probing behavior, we performed 17 16-h-long recordings with both males and females (nine males, eight females) on bromus plants, carried out each day from 10:00 to 02:00 hours (approximately 12 h of light per day; sunset occurring approximately at the 12th h of the EPG recording). A different plant was used for each insect.

Comparison of probing behavior on bromus, olive, and grapevine

Thereafter, we assessed the differences in *N. campestris* female probing behavior among the preferred host bromus, vs. grapevine (*Vitis vinifera* L., Vitaceae) cv. Cabernet Sauvignon and olive (*Olea europaea* L., Oleaceae) cv. Picual, through 6-h EPG recordings (11 records for bromus, 10 for grapevine, 11 for olive). The woody hosts were 3-month-old cuttings grown in 3-l pots filled with a mixture of soil: sand: vermiculite (6:2:3) in an insect-proof greenhouse (22–28 °C and 50–70% r.h.). We used only females due to scarcity of males within the rearing when the EPG-assisted host preference test was conducted, thus avoiding possible sex-related differences as reported by Cornara et al. (2018b) for *P. spumarius* on olive.

Electrical penetration graphs: tethering, recording, and data acquisition

Insects tethering, connection to the amplifier probes, recording, data acquisition, and analysis were performed

according to the protocol described for *P. spumarius* by Cornara et al. (2018b). Six-h EPG recordings were performed inside a Faraday cage supplemented with artificial light (220–240 V, 50 Hz, 815 lm). Sixteen-h recordings were also carried out under natural light coming from windows surrounding the Faraday cage, thus not supplementing the cage with artificial light, at 24 ± 3 °C and ca. 50% r.h. A single insect-plant combination was used for each recording. For the host plant comparison, during each 6-h EPG recording, we observed the probing behavior of *N. campestris* on the three plant species at the same time, with two replicates (i.e., two recorded insects and two EPG channels) per host plant. EPG channels used for the various host plants were alternated at each recording to prevent a positional effect. Probing behavior was recorded with a direct current (DC) EPG device, model Giga-8 (EPG-systems, Wageningen, The Netherlands) at 1 Giga Ohm input resistance. Output from the EPG at $100\times$ gain was digitalized at a rate of 100 samples per s per channel, and recorded using Stylet+ software (EPG-systems). Substrate voltage was adjusted following the calibration instructions of the DC-EPG equipment so that EPG output signals fit into the +5V to –5V window provided by the software.

Statistical analysis

Statistical analysis was carried out in R (R Core Development Team, 2019). The Shapiro–Wilk test indicated that the EPG data were not normally distributed (not shown). Therefore, we first investigated host-related and sex-related differences in the main EPG variables through non-parametric MANOVA (npmv) (Ellis et al., 2017), package ‘npmv’ (Burchett et al., 2017), both in the non-sequential variables [total waveforms duration per individual (WDI); total number of waveforms events per individual (NWEI); total number of probes with xylem ingestion (successful); and total number of probes without xylem ingestion (unsuccessful)], and in the sequential variables (time from the beginning of the recording to the first xylem contact; time from the beginning of the recording to the first xylem ingestion; time from the first probe to the first xylem contact; time from the first probe to the first xylem ingestion; time from the beginning of the recording to the first xylem ingestion >10 min; time from the first probe to the first xylem ingestion >10 min). Briefly, considering for example WDI, we created a matrix encompassing each of the waveforms representing specific probing activities: total duration of non-probing (np-WDI), pathway (C-WDI), xylem contact (Xc-WDI), xylem ingestion (Xi-WDI), interruption during xylem activities (N-WDI), and resting (R-WDI), as the duration of each waveform is theoretically correlated with the

others. Through npmv we assessed differences in WDI of the different waveforms/behavioral patterns among the plants tested (bromus, olive, and grapevine) and between sexes. Non-parametric MANOVA, besides indications on the occurrence of significant differences in waveforms duration (the matrix ‘WDI’), also provides tendencies observed in the duration of each of the six waveforms in terms of probability on each host/sex. The same approach was followed for NWEI, probes (both, number of probes containing xylem ingestion and probes without ingestion), and sequential variables (described above). A detailed description of npmv analysis can be found in Ellis et al. (2017).

In addition, for each EPG variable [e.g., duration of Xi (Xi-WDI)], and for the time spent by each individual in xylem ingestion in relation to the total probing time (Xi-WDI/total probing time), a Kruskal–Wallis non-parametric test was carried out. Dunn Test with Bonferroni adjustment (‘FSA’ package; Ogle et al., 2019) was used for pairwise comparison of the EPG variables among the three host plants tested.

To understand whether there were any changes or fluctuations in the feeding behavior of *N. campestris* during the day, we analyzed spittlebug hourly activities during the 16-h EPG recording. Hourly variations of probing behavior (WDI and NWEI) during the 16-h records were analyzed by nmpv. In addition, we performed generalized additive model (GAM), package ‘mgcv’ (Wood, 2017), on single variables WDI and NWEI for each EPG waveform, using the recording time (hour) as smoother. Graphs were generated using the package ‘ggplot2’ (Wickham, 2016).

Results

Neophilaenus campestris showed almost the same main EPG patterns and sequence of events previously described for *P. spumarius* (see Cornara et al., 2018b). No sex-related differences in probing behavior of *N. campestris* on bromus were found. However, we observed in addition to the interruption N, another kind of interruption of xylem activities not previously described in *P. spumarius*, provisionally called N2 (Figure 1). The waveform is characterized by a sudden voltage increase, followed by a gradual decrease, and is likely composed of four sub-phases of emf and emf/R origins, as determined by comparing positive vs. negative voltage records (Figure 1; Table 1). The pattern was present in ca. half of the recordings performed on bromus and grapevine (4/9 males in bromus, 5/11 females in bromus, 4/10 females on grapevine), but absent in individuals on olive (0/11). In this work, we marked and calculated the N2 as regular xylem activity interruptions N; however, further research and histological work is needed

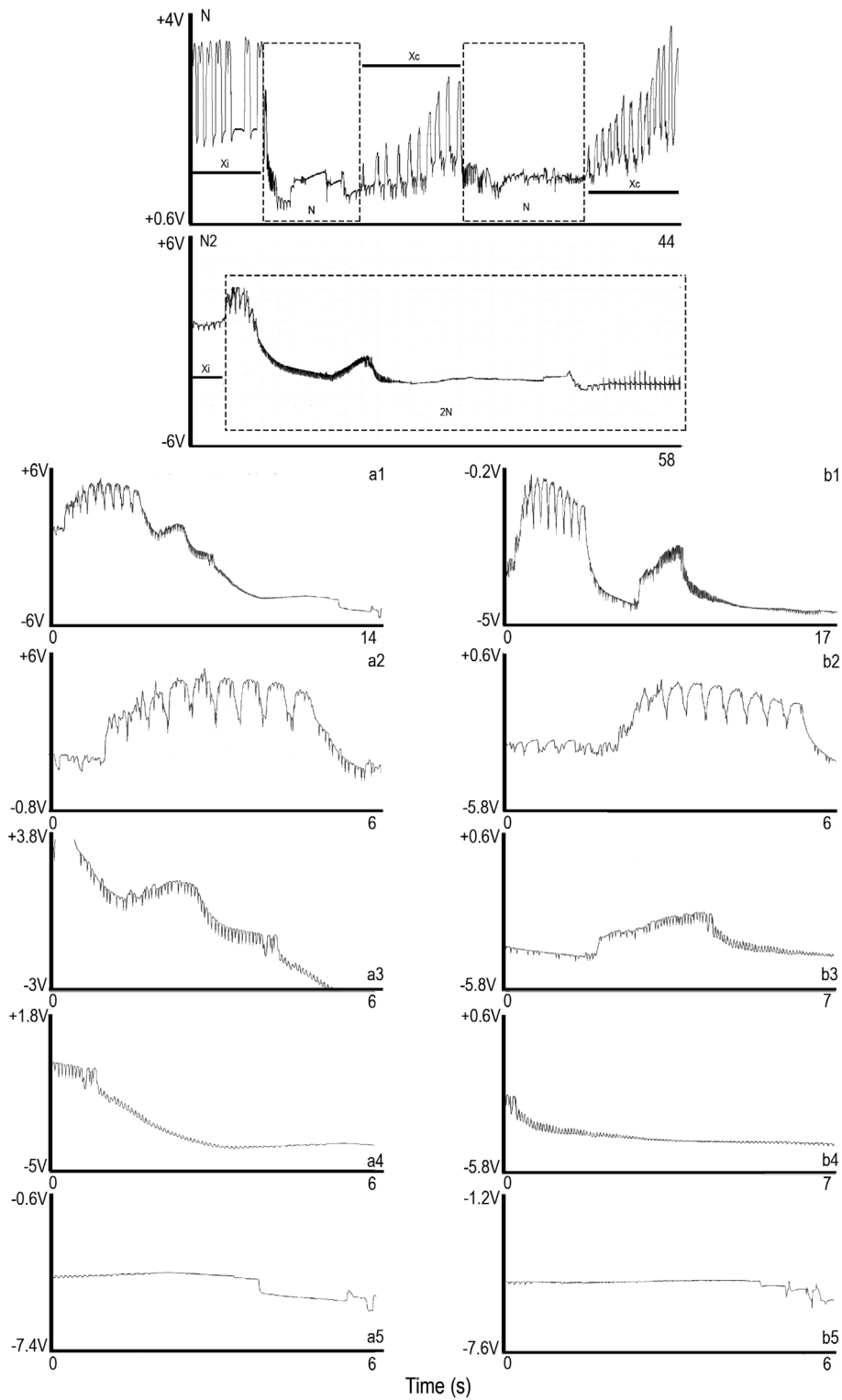


Figure 1 Waveforms N (top; two N waveforms are displayed, in dashed boxes) and N2 (down, in a dashed rectangle). The lower panels represent N2 recorded at positive voltage on the left (a1 is a general N2 view, a2–a5 represent one of the four identified sub-phases) and at negative voltage on the right (b1 is a general 2nd N view, b2–b5 represents one of the four identified sub-phases).

Table 1 Main electrical characteristics of the N2 waveform

Amplitude (%)	Frequency (Hz) (min-max)	Voltage level ¹	Electrical origin
95.89 (4.8 V)	Phase 1	5.42 (1-12)	e/i
	Phase 2	9.92 (3-17)	emf/R
	Phase 3	7.74 (0.1-16)	R
	Phase 4	1.31 (0.1-9)	R

5V = 100% amplitude.

¹e, extracellular; i, intracellular.

to deeply characterize the various sub-phases and to understand the biological meaning of this 'new' waveform.

Considering the development over time of *N. campestris* behavior during the 16-h recording on bromus (10:00–02:00 hours), according to npmv analysis, significant differences in hourly activities were observed for both WDI matrix (explained above, in 'Materials and methods') ($\Lambda = 1.39$; permutation test: $P = 0.01$) and the NWEI matrix ($\Lambda = 1.47$; permutation test: $P < 0.01$) (results from the npmv analysis and the tendency observed in the data in terms of probability are reported in Table S1). According to the GAM model, hourly differences in waveforms duration (WDI) were significant for non-probing ($F_{5,57,6,71} = 2.49$, $P = 0.01$), pathway ($F_{6,19,7,34} = 4.50$, $P < 0.01$), and resting ($F_{1,1} = 10.76$, $P < 0.01$). Differences in number of events (NWEI) were instead significant for non-probing ($F_{3,3,72} = 5.17$, $P < 0.01$), pathway ($F_{2,49,3,10} = 5.33$, $P < 0.01$), and xylem activities interruption ($F_{1,1} = 12.66$, $P < 0.01$). Overall, non-probing, pathway, and xylem interruption events (both number and duration) were concentrated in the first hours, except for peaks in duration of Xc and Xi during the central hours of the recordings (6th to 10th hour of the EPG recording approximately); the duration of resting tended to increase proportionally with the recording time (Figures 2 and 3).

According to the results of non-parametric MANOVA, we found a significant difference in *N. campestris* probing behavior between bromus, and both grapevine and olive (the latter overall similar) regarding the total waveform duration per individual WDI matrix ($\Lambda = 2.53$; permutation test: $P = 0.01$), and number of waveform events per individual NWEI matrix ($\Lambda = 2.19$; permutation test: $P = 0.03$). Considering the median values and the npmv model tendencies observed in the data in terms of probability (Table S2), of particular relevance were the differences in the duration of non-probing (bromus = 57.4 min, grapevine = 208 min, olive = 256.45 min), xy-lem ingestion (bromus = 165.2 min, grapevine = 109.5min, olive = 42.25 min), and resting (bromus = 55.3min, grapevine = 0.55 min, olive = 2.3 min) (Figure 4).

On the other hand, for the specific EPG variables, there were significant differences only for (1) duration of np, which was shorter in bromus than in olive ($z = -2.56$, $P = 0.03$), and similar between bromus and grape, and (2) duration of R, which was far longer in bromus than in both grapevine ($z = 3.43$, $P < 0.01$) and olive ($z = 3.13$, $P < 0.01$), and similar in grapevine and olive. In addition, the time spent in xylem ingestion expressed as percentage of the total probing time (same host-suitability indicator used for spittlebugs by Sandanayaka et al., 2013) was significantly longer in grapevine (82.8%) than in olive (41.1%) ($z = 2.10$, $P = 0.03$), whereas no significant difference was observed between grape and bromus (61.3%). Considering the Dunn test results for NWEI, number of R events was greater in bromus than in both grape ($z = 2.41$, $P = 0.04$) and olive ($z = 2.86$, $P = 0.01$). However, npmv model tendencies observed in the data in terms of probability indicate, besides R, relevant differences in NWEI of N, with the highest number of N events occurring in grapevine, whereas olive and bromus had similar values (median values: bromus = 1.9, olive = 2, grape = 4) (Figure 5).

For successful (with xylem ingestion) and unsuccessful (without xylem ingestion) probes, only the difference between bromus and grapevine was close to significance (np-MANOVA: $\Lambda = 2.66$; permutation test: $P = 0.05$) (Table S2). Considering median values, *N. campestris* on grapevine made slightly more probes with xylem ingestion (bromus = 2, grape = 2.5, olive = 1) and fewer probes without ingestion (bromus = 4, grape = 1, olive = 2) (Figure 6). Probes without xylem ingestion (unsuccessful) were significantly less numerous in grapevine compared to bromus ($z = 2.46$, $P = 0.04$), and similar between bromus and olive. No differences in sequential EPG variables were observed among tested plants.

Discussion

Overall, the probing behavior of *N. campestris*, that is, the main electrical pattern, does not differ substantially from that of *P. spumarius*, with the exception of a second type of interruption of the xylem activities, provisionally termed N2, previously not recorded in *P. spumarius*. The first part of the N2 resembles the non-pathway-like interruption described by Chuche et al. (2017) for *Scaphoideus titanus* Ball, and Stafford & Walker (2009) for *Circulifer tenellus* (Baker), and suggested to represent salivation activity during ingestion. Interestingly, N2 was only present on bromus and grapevine, suitable hosts according to the behavioral patterns observed (discussed below), but absent on olive, the least suitable among the plants tested. As well as for the previously described N, the biological meaning

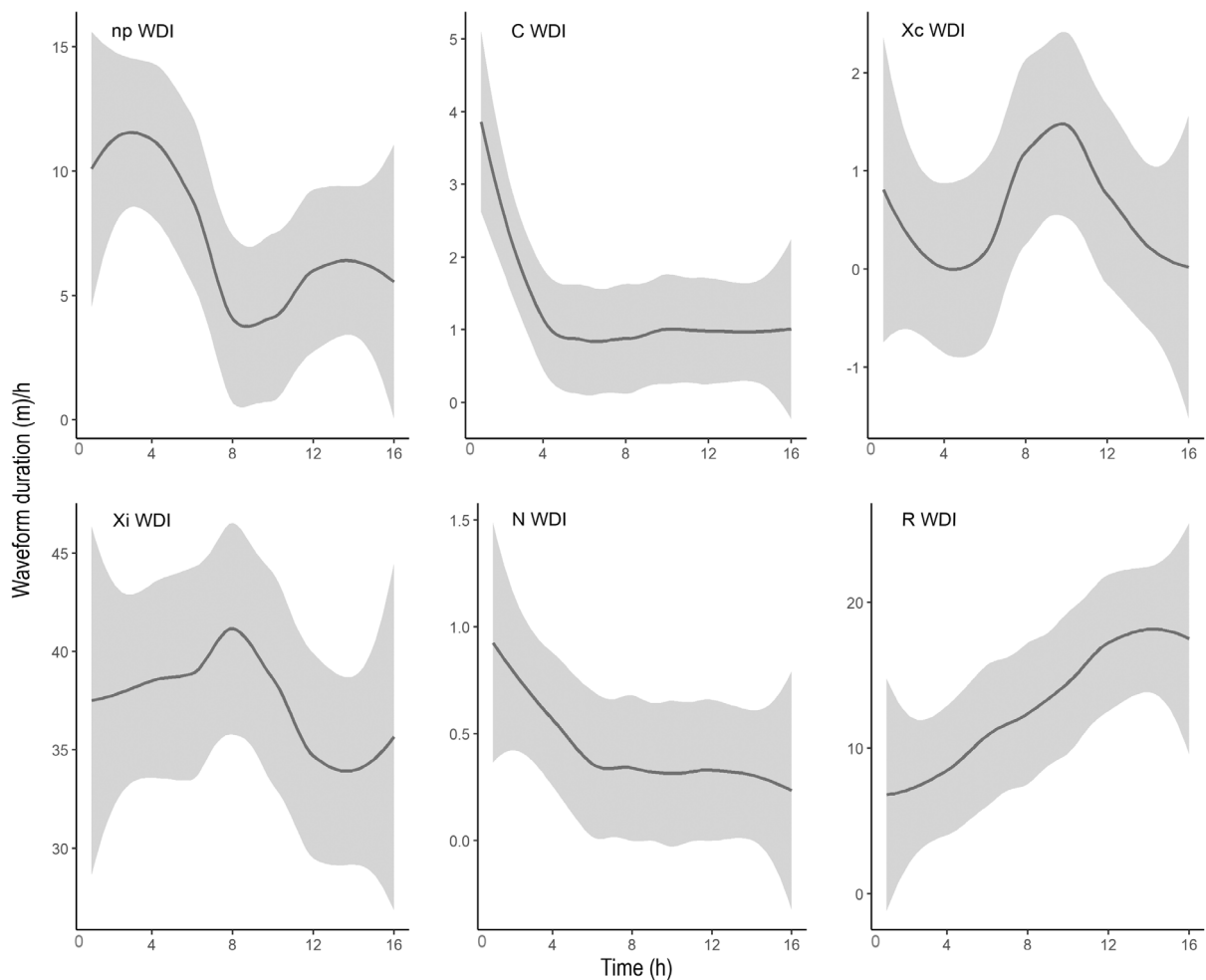


Figure 2 Total waveform duration (min) per insect per hour (WDI) during 16 h (10:00 to 02:00 hours) in *Neophilaenus campestris* males and females on bromus (np, non-probing; C, pathway; Xc, xylem contact; Xi, xylem ingestion; N, non-pathway interruption; R, resting).

of the waveform is currently unknown, and deserves dedicated studies. Waveform Xe, recently suggested by Cornara et al. (2020) as possible *X. fastidiosa* inoculation behavior, was only observed once for a single male on bromus. Given Xe was reported as occasional and rare event, more observations coupled with transmission experiments are needed to exactly determine the frequency of this behavior, and thus the probability of occurrence of putatively associated *X. fastidiosa* inoculation events, on grapevine and olive.

Significant hourly variation was observed in the 16-h records, particularly during the first hours when the insects performed more and longer activities associated with settling on the host plant (np, pathway C, and N), whereas resting duration tended to gradually increase with time. However, considering the datasets and the gam models, xylem contact and ingestion activities (in terms of WDI)

showed peaks in the central part of the recording, approximately from the 6th to the 10th hour. In our opinion, in accordance with Beck (1968) and Brodbeck & Andersen (1993) such peaks could be associated with synchronization of the spittlebug feeding activity with fluctuations in bromus xylem fluid chemistry, rather than endogenous insect factors regulating insect photoperiod activity. However, more research is needed to precisely correlate the insect's trophic activity and host acceptance with daily and seasonal variation in xylem chemistry and physiology, in order to eventually understand the factors driving spittlebugs host switching and dispersal.

The most interesting findings of our EPG study in the light of *X. fastidiosa* epidemiology regard the observed differences in *N. campestris* probing behavior between olive, a transient unsuitable host plant where the spittlebug is occasionally collected during the period of summer

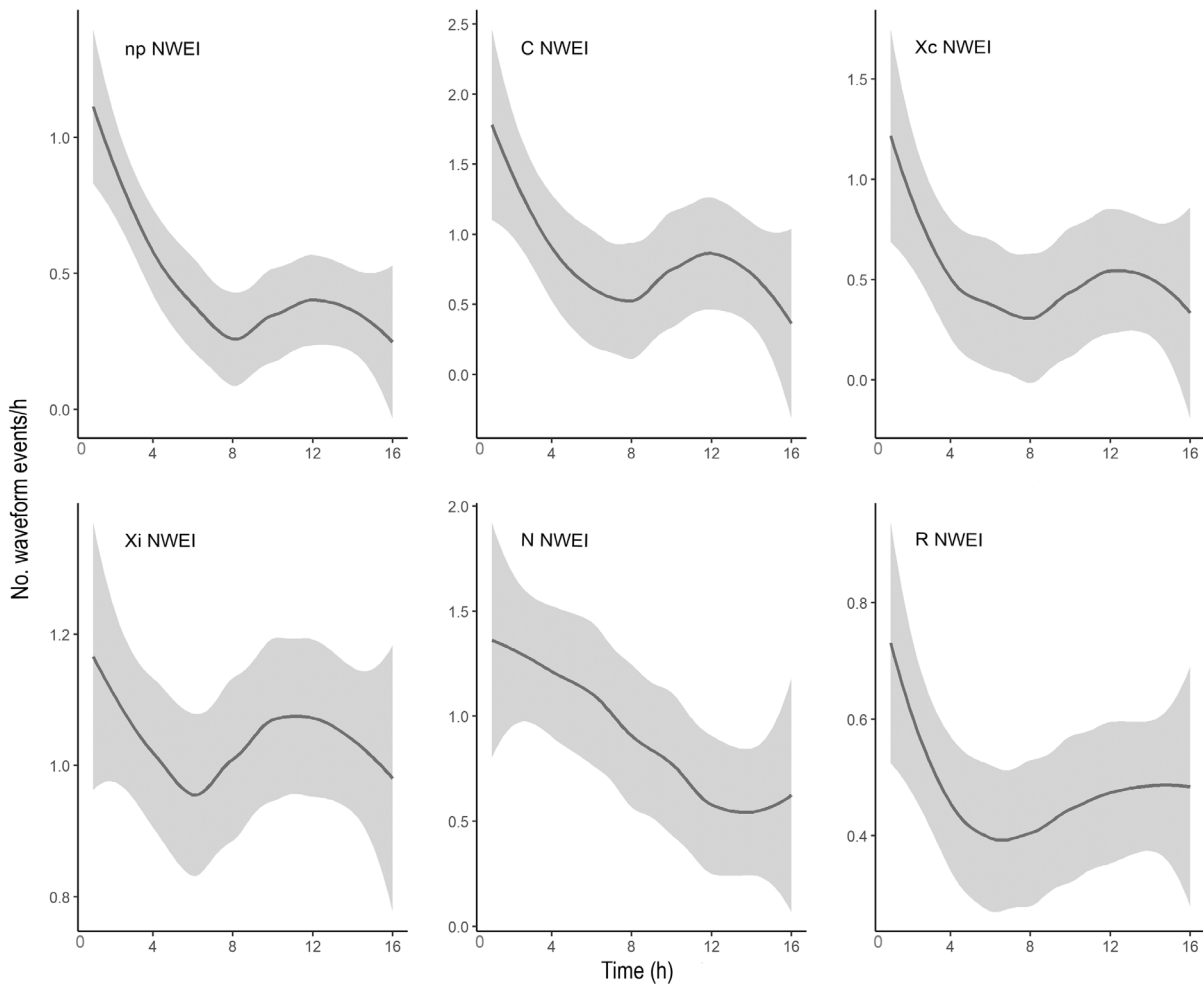


Figure 3 Number of waveform events per insect per hour (NWEI) during 16 h (10:00 to 02:00 hours) in *Neophilaenus campestris* males and females on bromus (np, non-probing; C, pathway; Xc, xylem contact; Xi, xylem ingestion; N, non-pathway interruption; R, resting).

dispersal (Cornara et al., 2017; Bodino et al., 2020), and two suitable hosts such as bromus and grapevine. *Neophilaenus campestris* spent a shorter time in non-probing activities on bromus compared to both grapevine and olive. A longer time spent resting on bromus compared to olive and grapevine could be related to physiological and anatomical differences between herbaceous and woody hosts.

However, when considering time spent on xylem ingestion as a percentage of the total probing time, a reliable indicator of host suitability according to Sandanayaka et al. (2013), grapevine was the most suitable host for the spittlebug, with around 82% of the total probing time spent in xylem ingestion. The value displayed on grape did not significantly diverge from the one observed on bromus, although the percentage on the latter host dropped

to ca. 60%, with also longer time spent on resting behavior. Olive was instead a less suitable host compared to grapevine, and overall, looking at xylem ingestion, the least suitable among the plant species tested.

Our observations were supported by field data, with bromus as one of the most common spittlebug adults' host upon emergence (Morente et al., 2018a; Bodino et al., 2020), and several ongoing and published reports about the insect's presence on vine canopies (Arrarás-Oroz et al., 2018; Morente et al., 2019; A Markheiser, unpubl; G Santoiemma, pers. comm.). In contrast, *N. campestris* is just occasionally collected on olive canopies during the adults' dispersal toward overwintering hosts (Cornara et al., 2017; Morente et al., 2018a; Bodino et al., 2020). In addition, as testified by the relatively low number of probes without xylem ingestion compared to both bromus and

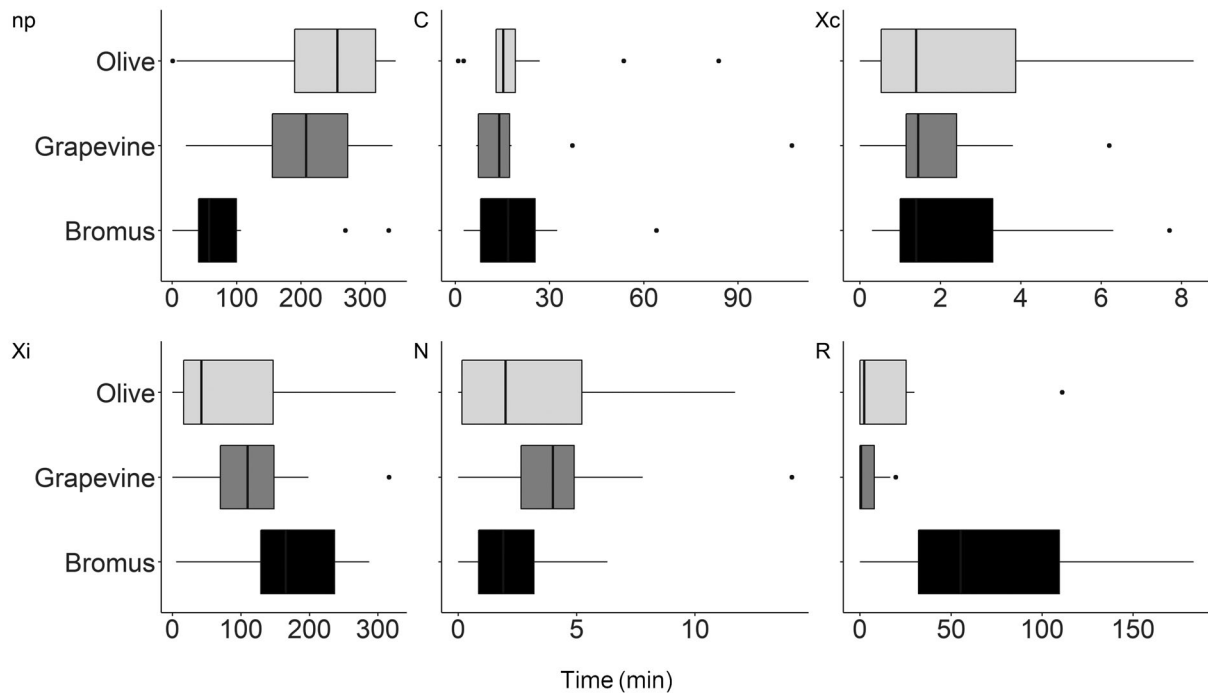


Figure 4 Total waveform duration (min) per insect (WDI) in *Neophilaenus campestris* females on bromus, grapevine, and olive (np, non-probing; C, pathway; Xc, xylem contact; Xi, xylem ingestion; N, non-pathway interruption; R, resting). The boxplots show the median value and the first and third quartile (upper and lower box). The whiskers show 1.5× the interquartile range. The dots are outliers.

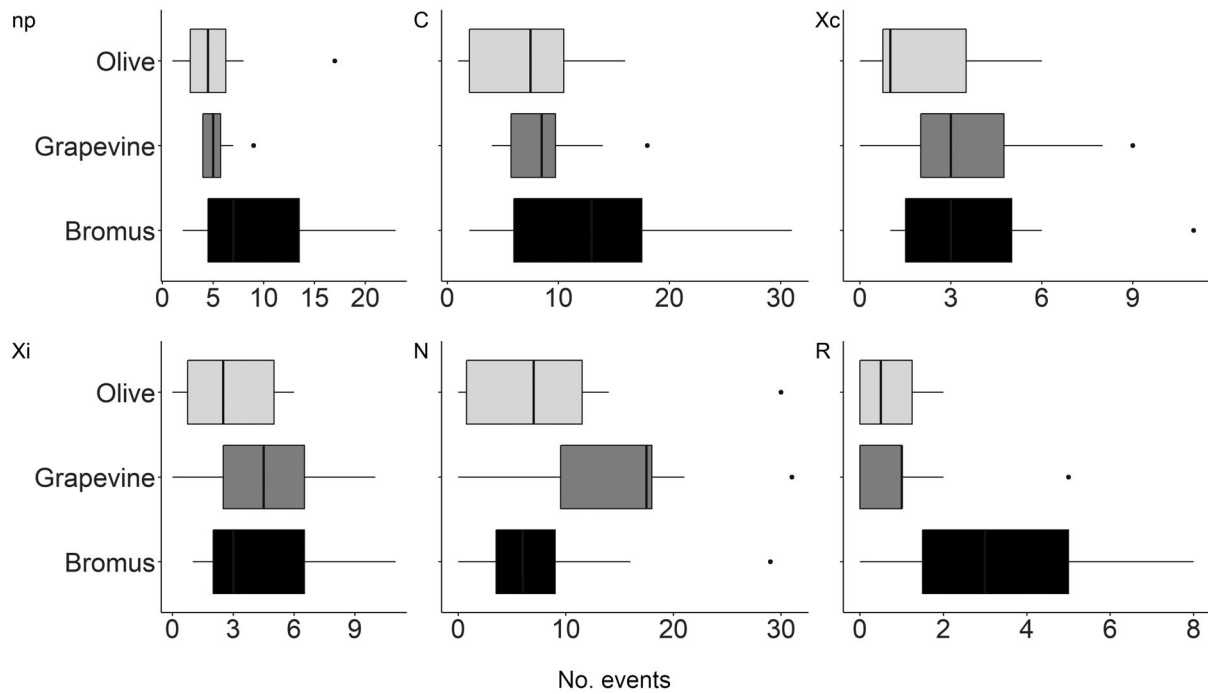


Figure 5 Number of waveform events per insect (NWEI) in *Neophilaenus campestris* females on bromus, grapevine, and olive (np, non-probing; C, pathway; Xc, xylem contact; Xi, xylem ingestion; N, non-pathway interruption; R, resting). The boxplots show the median value and the first and third quartile (upper and lower box). The whiskers show 1.5× the interquartile range. The dots are outliers.

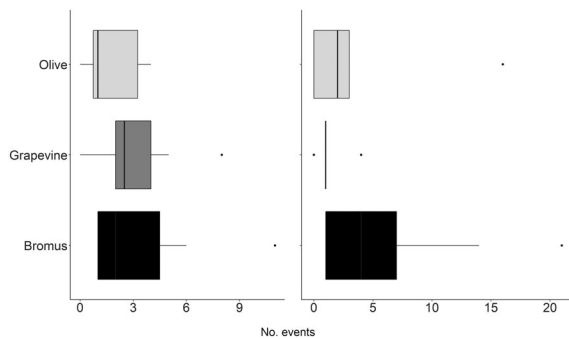


Figure 6 Successful (left) and unsuccessful (right) number of probes in *Neophilaenus campestris* females on bromus, grapevine, and olive (np, non-probing; C, pathway; Xc, xylem contact; Xi, xylem ingestion; N, non-pathway interruption; R, resting). The boxplots show the median value and the first and third quartile (upper and lower box). The whiskers show $1.5 \times$ the interquartile range. The dots are outliers.

olive, xylem vessels in grapevine could be easily accessible to the spittlebug. Therefore, when ground cover is no longer suitable for spittlebug adults, the individuals might find a favorable host in grapevine.

Furthermore, *N. campestris* was found to be a competent vector of *X. fastidiosa* to olive under experimental conditions, although with an extremely low transmission efficiency (one infected plant out of 20 exposed to five insects each for an IAP of 96 h) (Cavaliere et al., 2019). This might be explained considering the low numbers of probes with xylem activities (successful probes) observed in the present study on olive, given the bacterium transmission is positively correlated with the number of times the vector enters in contact with a xylem vessel (Jackson et al., 2008; Daugherty & Almeida, 2009; Cornara et al., 2020). In contrast, successful probes occurred $2.5 \times$ more on grapevine than on olive, suggesting a higher probability of occurrence of inoculation events on grape. Such hypotheses should be tested by coupling classical and EPG-assisted *X. fastidiosa* transmission tests with behavioral and ecological studies, in order to broaden our knowledge on *X. fastidiosa*-pathosystems in Europe, currently limited to spring and summer secondary transmission of the bacterium to olive mediated by *P. spumarius*.

In addition to contributing to the knowledge of European candidate vectors of *X. fastidiosa* probing behavior, a secondary aim of the present paper was to further stress the current difficulty in interpreting the outcomes of EPG studies on spittlebugs. Raw data and tendencies observed in npmv were only partly confirmed by Kruskal–Wallis tests followed by pairwise comparisons among the three host plants tested, an approach widely used in EPG studies. However, the main problem related to EPG data analysis,

especially with spittlebugs is the extreme variability in probing behavioral patterns observed in studies performed in Europe to date (Cornara et al., 2018b; Markheiser et al., 2020), with frequent outliers biasing the analysis outcomes. Larger sample sizes (at least 15–20 records per treatment) could resolve the ‘variability’ issue, although in our experience spittlebugs are very variable in their probing behavior and data tend to be overdispersed even using sample sizes of >20 individuals per treatment. Spittlebugs are also difficult to handle compared, for example, to aphids and EPG experiment with this taxon are particularly costly and time-consuming (considering also the records to be discarded for problems of connection or poor signal quality). In contrast, EPG-assisted observations should be concentrated over a period as short as possible to avoid differences among the replicates related to the age of the insects to be tested (Walker, 2000). We therefore propose the combined approach used in the present study, that is, multivariate analysis of waveform duration, number of waveform events, number of probes, and sequential variables, followed by univariate analysis on single EPG variables, and graphic interpretation of data distribution and median values, as a possibly more comprehensive way to interpret the outcomes of EPG-assisted probing behavioral studies overcoming strict single-model ‘P-value based’ conclusions. However, when possible, large sample sizes with 20 or more replicates per treatment are highly recommended to reduce the effect of outliers and uncertainties in data interpretation. Another issue related to EPG studies on spittlebugs is the procedure followed for discriminating and marking the different waveforms. Knowledge on spittlebug behavior and the biological meaning of EPG waveforms considerably lags behind other sap-sucking insects such as aphids or sharpshooters (Backus & Shih, 2020). Although the main electrical patterns have been identified and described at least for *P. spumarius* (Cornara et al., 2018b), their biological meaning is mostly uncertain (with the exception of xylem ingestion); in addition, a standardized EPG recordings marking protocol is urgently required, accounting for the high variability in spittlebug behavior, and making studies performed by different groups under different conditions comparable.

In summary, data obtained from our probing and feeding behavior observations, coupled with field reports, suggest *N. campestris* should be taken into consideration as a potential vector of *X. fastidiosa* to grapevine. We suggest that, particularly in areas where strains belonging to subsp. *fastidiosa* have been introduced, such as the Balearic islands (EFSA, 2019), extensive research effort should be put in place to characterize the spittlebug population dynamics and investigate the transmission competence of

N. campestris and its role in bacterium epidemiology in vineyards.

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Author Contribution

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Non-parametric MANOVA results for WDI (waveform duration per individual) and NWEI (number of waveform events per individual) in 16-h recordings on bromus.

Table S2 Non-parametric MANOVA results for WDI, NWEI and probes (successful and non-successful) in 6-h recordings on bromus, olive, and grapevine.