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Temporal dynamics of the transmission of *Xylella fastidiosa* subsp. *pauca* by *Philaenus spumarius* to olive plants

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With 7 figures, 2 tables and 3 supplements

Abstract: The spittlebug *Philaenus spumarius* L. (Hemiptera: Aphrophoridae) is the predominant vector of *Xylella fastidiosa* Wells et al. (Xanthomonadales: *Xanthomonadaceae*) (*Xf*) to olive trees in the Apulia Region in Italy. Previous studies focused on assessing the *Xf* transmission efficiency of spittlebugs and the natural infectivity of the *P. spumarius* populations. However, the factors that influence *Xf* transmission by *P. spumarius* to olive are still largely unknown, and these knowledge gaps hamper the comprehension of the epidemiology of emerging *Xf*-associated diseases. We have performed two sets of experiments to study the transmission biology of *Xf* by *P. spumarius* to understand the kinetics of the bacterial persistence, transmission efficiency and the spread rate of *Xf* among olive trees in summer and autumn. The results show that i) *P. spumarius* is a competent *Xf* vector to olive plants throughout its adult life, ii) the bacterial load in the foregut of the vector increases during the first 2-3 weeks after acquisition and then becomes stable, iii) the transmission rates may vary significantly during the year and under different climatic conditions, and iv) the differential survival of vectors – which is influenced by the age of the insects, the season and climatic conditions – may affect the spread of *Xf* in olive plants. These results will help to improve both the modelling of the spread of the pathogen, by allowing the effect of the insect vectors to be explicitly incorporated, and the design of effective control and prevention measures against this vector-borne disease.

Keywords: vector-borne disease, vector bacterial load, Olive Quick Decline Syndrome, OQDS, Aphrophoridae, spittlebugs, non circulative-persistent transmission

1 Introduction

Transmission biology is of major importance to outline the disease epidemiology of insect-borne plant pathogens (Jeger et al. 1998; Daugherty & Almeida 2009), and its detailed knowledge is key to elaborating effective disease management strategies (Almeida et al. 2005). The transmission of pathogens by insect vectors is a complex process, characterised by several steps and actors, which involves different areas of knowledge, including molecular biology, physics, physiology and ecology (Killiny et al. 2009; Sicard et al. 2018; Ranieri et al. 2020; Backus & Shih 2020). Ultimately, the most fundamental characteristic of vector transmission – especially from a disease control perspective – is efficiency, i.e. how often a vector transmits a pathogen over time or per transmission opportunity (Purcell & Almeida 2005). The transmission efficiency of persistent plant pathogens by insect vectors may vary significantly over time after acquisition, and it is a process that is influenced by the condition of the host and environmental factors (Anhalt & Almeida 2008; Daugherty et al. 2009; Ghanim 2014; Daugherty & Almeida 2019). Nonetheless, epidemiological models rarely explicitly include these aspects, often because of a lack of reliable estimates of the key parameters of the vector transmission of

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pathogens. These limitations lead to biases in the outcomes and in the predictions of disease dynamics models (Jeger 2000; Jeger & Bragard 2018; Allen et al. 2019).

The meadow spittlebug, *Philaenus spumarius* L. (Hemiptera: Aphrophoridae), is a key vector of the bacterium *Xylella fastidiosa* Wells et al. (Xanthomonadales: *Xanthomonadaceae*) (*Xf* hereafter) in Italy and Europe (Cornara et al. 2018, 2019). *Xf* subsp. *pauca* strain ST53 is associated with the most severe *Xf*-epidemic that is currently affecting Europe, being the causal agent of the olive quick decline syndrome (OQDS) in Apulia (South Italy) (Saponari et al. 2019). In this region, this introduced bacterium has encountered both large populations of a previously neglected vector, the xylem-sap feeder insect *P. spumarius*, and an agricultural landscape dominated by susceptible olive plants, which in turn has led to a dramatic epidemic that has wiped out the olive production in the infected area in less than one decade (Saponari et al. 2019; Schneider et al. 2020). Ongoing research on *Xf* in Europe has revealed that *P. spumarius*: i) is the dominant xylem-sap feeder in olive agroecosystems in the Mediterranean region (Ben Moussa et al. 2016; Morente et al. 2018; Antonatos et al. 2019; Dongiovanni et al. 2019; Bodino et al. 2019), ii) adults are found naturally infected with *Xf* in olive groves during summer and autumn (Cornara et al. 2017b; Cavalieri et al. 2019), and iii) it is able to transmit the bacterium from olive-to-olive and from other plants to olive plants in laboratory trials (Cornara et al. 2017a, 2020; Cavalieri et al. 2019).

The current knowledge on *Xf* transmission biology has been obtained from American pathosystems – e.g. grapevine and citrus diseases – where sharpshooters (Hemiptera: Cicadellidae: Cicadellinae) represent the main group of vectors (Redak et al. 2004; Esteves et al. 2019; Cornara et al. 2019). Once acquired through feeding in xylem vessels colonised by *Xf*, bacterial cells multiply in the foregut of the insect vector and are persistently transmitted in a noncirculative manner, with no transstadial or transovarial passage and no apparent latent period (Severin 1950; Freitag 1951; Purcell & Finlay 1979). The acquisition and inoculation efficiency of *Xf* may vary to a great extent, as a result of several factors – e.g. vector species (Daugherty & Almeida 2009; Marucci et al. 2008), bacterial strain or genotype (Esteves et al. 2019), source or recipient plant species and tissue, conditions of the host plants (water stress, disease symptoms) (Daugherty et al. 2011; Krugner et al. 2012; Krugner & Backus 2014), season and climatic conditions (Daugherty et al. 2009, 2017) – all of which can shape the vectors' feeding preferences and acquisition rates (Almeida et al. 2005; Gruber & Daugherty 2013).

Despite its importance in affecting epidemics spread and severity, several aspects of the transmission biology of *Xf* by *P. spumarius* on olive trees have not yet been investigated, since the previous studies on the Apulian pathosystem focused primarily on assessing the transmission capability of spittlebugs. In addition, some information inferred from the American pathosystems may not be directly transferable to the Italian olive disease, given the differences (e.g. vector species, *Xf* genotypes, host plants) that affect the transmission parameters and inoculation efficiency (e.g. Almeida et al. 2005; Esteves et al. 2019). A better knowledge of *Xf* transmission parameters of European vectors is therefore necessary, in order to allow epidemic models to consider the transmission dynamics of the pathogen, which, so far, has not been explicitly incorporated in any of the proposed models of the spread of *Xf* in Europe (White et al. 2017, 2020; Strona et al. 2020).

The aim of this work was to study the kinetics of *Xf* subsp. *pauca* ST53 in *P. spumarius* over time and the transmission efficiency of *Xf* by *P. spumarius* to olive trees throughout the year. We have investigated how the acquisition, bacterial load and transmission efficiency are affected by the season, climatic conditions, and the duration of plant exposure to infectious vectors. More specifically, we carried out experiments at different times of the year to determine i) *Xf* persistence, and the size of the population within the foregut of *P. spumarius* at different intervals from the access to the infected source plant, ii) the transmission rate to olive seedlings after different post acquisition periods, and iii) the spread of *Xf* by infected spittlebugs within an experimental population of olive seedlings under different seasonal and climatic conditions.

2 Materials and methods

2.1 Insects and plants

P. spumarius adults were field collected in dry meadows and olive groves located in *Xf*-free areas in northern Bari province (approx. 41.111° N, 16.519° E; Ruvo di Puglia (BA), Apulia, Italy) one to two weeks prior to the start of each experimental assay. The location of insect collection was about 120 km from the closest point of infected area at the time of the experiment. Collected insects were maintained in mesh and plastic fabric cages (Bugdorm: 75×75×115 cm) containing potted host plants (*Medicago sativa, Vitis vinifera, Sonchus oleraceus, Vicia faba, Erigeron* spp.). The cages were placed in a climatic chamber (24.7 ± 1.0 °C, 72.1 ± 7.2% RH) in summer and outdoors, in a shaded location, during autumn in the "Li Foggi" nursery of ARIF (Agenzia Regionale Attività Irrigue e Forestali) (Gallipoli, province of Lecce). The air temperature and relative humidity were recorded hourly under both conditions using data loggers (HOBO U23-002; Onset Computer, Bourne, MA, USA). Adult mortality was about 5–10% of the total collected insects. To confirm the *Xf*-free status of the reared spittlebugs, since that a pre-screening on susceptible plants was not feasible given logistic constraints, 20–30 individuals were randomly collected from maintenance cages before starting each acquisition assay and individually tested by real time PCR (qPCR). No positive individuals were detected.

The recipient olive plants used for all the experiments consisted of certified pathogen-free olive seedlings produced and maintained in the Premultiplication Centre facilities at CRSFA. Seedlings of about 20–30 cm in height (6–8 months old) with active growing apexes were selected and transferred to the "Li Foggi" nursery of ARIF just before starting each trial.

Two different sets of experiments were carried out in order to gain information on the influence of the post-acquisition time, and of the seasonal and climatic conditions on transmission efficiency, persistence, and the multiplication of *Xf* in the vector, as well as on the survival of the insect vector.

2.2 Kinetics of *Xf* colonisation of *P. spumarius* and its transmission efficiency

The olive-to-olive transmission efficiency, and *Xf* retention and multiplication in *P. spumarius* adults were tested in four separate assays, carried out in different seasons during 2017 and 2018, in order to investigate the effects of time after the pathogen acquisition access period (hereafter AAP), of the season (summer: late June-July; autumn: late September-November) and of the age of the insects on the transmission parameters. The acquisition of *Xf* by *P. spumarius* adults was performed by caging the insects on branches of naturally infected olive trees (4–5 years old, cv. "Cellina di Nardò") located in olive groves in the municipalities of Gallipoli and Parabita (province of Lecce). Branches considered suitable for acquisition were first tested by qPCR (approx. two weeks before the AAP) to assess for the presence and abundance of *Xf* in young flushes. The source plants hosted bacterial populations ranging from a median value of 8.99×10^5 [interquartile range (hereafter IQR): $1.41 \times 10^5 – 1.99 \times 10^6$] CFU/g in summer to 1.88×10^5 (IQR: $1.22 \times 10^5 – 2.41 \times 10^5$) CFU/g in autumn. When considering the young and mature leaves from the branches selected for acquisition separately, the median bacterial populations ranged from of 2.06×10^5 (IQR: $7.69 \times 10^4 – 9.15 \times 10^5$) CFU/g of tissue in young leaves to 1.5×10^6 (IQR: $5.06 \times 10^5 – 3.98 \times 10^6$) in mature leaves. *Philaenus spumarius* adults coming from the maintenance cages were isolated, i.e. enclosed in mesh sleeves, on a total of 10–12 *Xf*-positive branches of 6–12 field-grown olives for an AAP of three days (72h), in groups of 50–100 individuals per branch.

At the end of the AAP, spittlebug cohorts from different source plants were pooled and transferred, for a post-AAP maintenance period, to five new cages (Bugdorm: 75×75×115 and 45×45×90 cm), containing potted non-host plants of *Xf* subsp. *pauca* strain ST53 (*V. vinifera, S. oleraceus, V. faba, Pistacia lentiscus* and *Vicia sativa*) (EFSA 2018). Inoculation assays were carried out using insects randomly collected from post-AAP maintenance cages at different time points after the AAP (Supplementary Tab. 1), which were transferred to testing plants (i.e. olive seedlings) for an inoculation access period (hereafter IAP) of three days (72 h). Each replica consisted of one potted olive seedling enclosed in a mesh sleeve, on which five *P. spumarius* individuals were confined, and thus had access to the entire plant during IAP. Five replicas were carried out at each time point after the AAP, with a total of 25 insects for each inoculation assay. Post-AAP insects were collected from the maintenance cages at several time points and stored directly in ethanol, for qPCR estimation of the bacterial load (see Supplementary Tab. 1). The AAP and IAP durations (72h each) were chosen on the basis of both preliminary trials and previous studies (Cavalieri et al. 2019) in order to maximise the transmission efficiency and insect survival. Both IAP assays and post-AAP rearing took place in the climatic chamber located at the above-mentioned "Li Foggi" nursery at temperatures of 24.7±1.0 °C and 72.1±7.2 % RH in summer and 20.8±1.0 °C and 77.9±7.6 % RH in autumn, respectively. At the end of IAP, the live and dead insects were both collected, and stored individually in 90% ethanol at −20 °C. The insect survival rate was recorded for each AAP and IAP assay. The recipient plants were treated with a systemic insecticide (Imidacloprid, Confidor 200 SL) and kept in an insect-proof screenhouse. Olive test plants were analysed for *Xf* at both 6 and 12 months after the experimental assays. The plants that tested positive in the first assay (6 months) were always positive in the second assay (12 months).

2.3 Spread of *Xf* by *P. spumarius* under microcosm conditions

Experimental assays were carried out in microcosms to assess the effect of i) IAP duration, ii) the climatic conditions (semi-field vs controlled), and iii) the season on the spread of *Xf*-infection within an olive seedling population. The experiments were conducted in the same periods as the transmission kinetic assays, i.e. summer (July) and autumn (September–October) for two consecutive years (2017 and 2018). We followed the same procedure as the one described above for *Xf* acquisition by *P. spumarius* adults. At the end of the 72h AAP, insects were collected from olive branches, pooled in a single cage and randomly transferred to mesh cages with a wooden frame (microcosms) (93 × 47.5 × 47.5 cm) containing 16 olive seedlings each, positioned at regular spacing. Seventeen individuals were introduced into each microcosm in the 2017 assays, and 32 individuals in the 2018 assays. Spittlebugs were released, in the centre of the microcosm, using a Falcon tube, which was shaken gently until all the insects had left. To test the effect of climatic conditions, one set of cages (n = 12) was set up under controlled climatic conditions (summer trials (2017: 17/07 – 07/08; 2018: 22/06 – 16/07): 24.9 ± 1.9 °C, 74.5 ± 7.8% RH; autumn trials (2017: 24/09 – 15/10; 2018: 29/09 – 19/10): 22.6 ± 0.5 °C, 82.7 ± 6.5 % RH), while another set (n = 12) was set up in the same periods under semi-field climatic conditions, in a shadowed area inside the above-mentioned nursery (summer: 26.3 ± 5.40 °C, 64.4 ± 22.5 % RH; autumn: 17.5 ± 3.9 °C, 63.3 ± 4.2 % RH). The climatic

conditions were monitored, using dataloggers, by inserting probes into a randomly chosen microcosm. Insects were caged in each set of microcosms for different IAP durations: 3-7-14-21 days, each with three independent replicates (i.e. 3 single microcosms at each IAP for each climatic condition). The olive seedlings were watered every two days during the assays through individual drip irrigation, avoiding disturbances to insects. All the spittlebugs found inside the microcosms at the end of IAP, both dead and alive, were collected and stored in ethanol 90% at –20 °C. The insect survival rate was recorded for each microcosm at the end of the IAP. The recipient olive seedlings were then treated with imidacloprid and kept in an insect-proof screenhouse. The test plants were analysed for *X. fastidiosa* 6 and 12 months after the experimental assay. Plants testing positive in the first assay (6 months) were always positive in the second assay (12 months), when in average 30% more plants tested positive.

2.4 *Xf* detection in insects

The insect head was removed using needles and a stereomicroscope. Each head was then homogenised in CTAB buffer with a tungsten carbide bead (7mm in diameter), using a Mill300 mixer (Qiagen, Germany). Homogenised samples were incubated at 65°C for 30min prior to being treated with an equal volume of chloroform – isoamyl alcohol (24:1), followed by 2-propanol precipitation. The recovered pellet was eluted in 30µl of sterile water. One microlitre of the purified DNA was used for the real-time qPCR reactions (final volume of 12,5 µl), using the specific primers and the TaqMan probe described in Harper et al. (2010). A 10-fold serial dilution of *X. fastidiosa* cultured cells, from 10^6 CFU/ml to 10^2 CFU/ml, was included in all the assays to estimate the bacterial populations in the tested insect samples. The mean number of *Xf* cells in the amplified samples was automatically calculated by CFX Maestro TM Software (Bio-Rad). Samples yielding doubtful qPCR results were re-amplified in direct and nested PCR assays, targeting the *holC* gene, according to Cruaud et al. (2018). The samples that displayed the specific band on agarose gel were then considered positive.

2.5 *Xf* detection in plants

The source plants used for the field acquisition of *Xf* were tested by collecting leaves from the branches on which the insects were caged. Two leaf samples were collected from each plant: one, including 6–8 young leaves, collected from the apical part of the shoots (within the last 5–8cm) and the second one including 6–8 mature leaves collected from the basal part of the shoots. Petioles and midribs were obtained from each sample and homogenised in CTAB buffer (1:10 w:v). An aliquot of 1ml of the obtained plant sap was then processed following the standard CTAB protocol (EPPO 2019). Real time PCR reactions were set up for the detection and quantification of the bacterium in these samples, as previously described for the insects. The results of bacterium quantification in the two samples are reported above in the kinetic experiment description. In order to test the recipient plants, 4–6 leaves were detached from the seedlings and processed as described above. All the recipient plants were tested twice: a first screening was performed 6 months after the IAP (data not shown) and then a final assessment was made 12 months after the IAP. Only data from this final assessment were used for the statistical analysis.

2.6 Statistical analysis

The proportion of *Xf*-positive *P. spumarius* and the proportion of infected olive seedlings in the kinetic experiment were modelled separately by logistic GLMM (binomial link), with *Time post-acquisition, Season, Sex* and their interaction as the fixed covariates and *Year* as the random intercept. The size of *Xf* population in the infected *P. spumarius* individuals, i.e. the number of bacterial cells in insects' mouthparts, was analysed throughout the kinetic experiment and separately for each assay by both linear regression and Michaelis-Menten (M-M) (1) models:

$$y = \frac{ax}{(b + x)} \qquad (1)$$

where a and b are model coefficients, y represents the number of *Xf* cells in the insect head, and x represents the days after the beginning of AAP. The model fit was compared by Akaike's Information Criterion, corrected for small sample size (AIC_c). The size of the *Xf* population was decimal log transformed ($\log10(x + 1)$) in all the tests to meet the model assumptions. The proportion of infected olive plants was analysed by logistic GLM (binomial link) as a function of the fixed covariates *Mean Xf population size* in vector batch, *Number of Xf-positive individuals* in inoculation batch and *Season*. The efficiency of transmission by *P. spumarius*, i.e. inoculation rate, was estimated using two non-linear functions derived from binomial probability model (2) and Poisson probability model (3), respectively (Purcell 1981; Daugherty & Almeida 2009):

$$P_{NB} = 1 - (1 - ba)^{NB}, \qquad (2)$$
$$P_{NB} = 1 - e^{-baNB}, \qquad (3)$$

where N is the number of vectors, B is the IAP duration, a is the probability that a vector is infected, and b is the vector inoculation rate. N and B were fixed parameters in our study (5 insects, 3 days), and both a and P_{NB} were known for each vector batch, given that the insects and inoculated plants were individually PCR-tested for *Xf*. The constant b, i.e. vector inoculation rate, was the only parameter to be estimated, fitting (2) and (3) to the kinetic dataset using non-linear least-squares regression (*nls* function). We then compared the over-all fit of both models using Akaike's information criterion (AIC).

The proportion of *Xf*-positive *P. spumarius* in the spread rate experiment was only modelled for the 2018 assays, due to the low number of insects recaptured in the 2017 assays, with *Inoculum duration*, *Climatic conditions*, *Season*, and their interactions using binomial GLM (proportion). The size of the *Xf* population in insects (decimal log transformed) was analysed by ANOVA using both the full dataset (PCR-positive and -negative individuals) and a subset of PCR-positive insects only. The proportion of infected olive seedlings was analysed using the full dataset (2017 + 2018) with binomial GLMM with *Inoculum duration*, *Climatic conditions*, *Season*, *Year* and their interactions, with replica, i.e. a single microcosm, as random intercept. The survival rate of *P. spumarius*, i.e. the number of individuals alive at the end of IAP, was analysed by binomial GLMM as a function of the fixed covariates *Inoculation duration*, *Climatic condition* and *Season*, with *Year* as random intercept. All the analyses were performed in R 4.0.3 (R Core Team 2020), with *lme4* (Bates et al. 2015), *nlme* (Pinheiro et al. 2020), and *ggplot* (Wickham 2016) packages.

3 Results

3.1 Kinetics of *Xf* colonisation of *P. spumarius* and its transmission efficiency

The overall survival rate of the spittlebugs isolated after a 3-day IAP on olive seedlings was 77.1% (343 of 445 individuals), and the survival of *P. spumarius* isolated on olive seedlings was similar among the assays and for the post-AAP time periods, with an IQR of between 60% and 84%. The prevalence of *Xf*-positive insects varied at different time points after acquisition and between the assays. Overall, the cumulative diagnostic results at different post-AAP times showed a proportion of 50% of *Xf*-positive insects (276 positives on 553 tested). The interaction of the fixed effects Time post-AAP and Season on the proportion of *Xf*-infected *P. spumarius* was significant (Season × Time post-AAP: $\chi^2_1 = 8.03$, P = 0.004) (Tab. 1). In other words, the proportion of *Xf*-infected *P. spumarius* was higher in the autumn assays (55–83%) than in the summer ones (20–60%), during the first post-acquisition days (Fig. 1). The proportion of *Xf*-infected insects then decreased over time post-AAP in the autumn assays (down to 40–50%), while it remained quite stable or increased slightly in the summer assays (30–60%) during the whole post-AAP period, up to 78 days (Fig. 1). The two sexes had the same proportion of *Xf*-positive individuals [males = 0.455 (76/167), females = 0.541 (190/351)] ($\chi^2_1 = 0.52$, P = 0.471).

3.2 Bacterial load in the insects

The *Xf* populations in the foregut of *P. spumarius* varied mostly from a few hundred to a few thousand cells (n = 272; IQR: 70–3,500 cells/insect; median: 836 cells/insect). A total of 10% of positive insects (n = 29) hosted more than 10^4 cells in their mouthparts, with one extreme value of 1.2×10^5. The bacterial load of the infected *P. spumarius* was not constant, but increased significantly over time post-AAP, i.e. the slope parameter *a* of the M-M model was always significantly different from zero, and was greater in the autumn assays than in the summer ones (M-M model summer 2018: $a = 3.02 \pm 0.19$, $t = 15.61$, P < 0.001; M-M model autumn 2018: $a = 3.64 \pm 0.21$, $t = 17.58$, P < 0.001) (Fig. 2). The *Xf* load during the first days after acquisition, i.e. 3–9 days after the start of AAP, consisted in a few hundred cells (IQR: 70–600 cells/insect; median: 280 cells/insect). The *Xf* load increased over time, reaching approximately 10^3–10^4 cells per insect

Table 1. Estimated odds ratios, z-statistics significance and confidence interval (95%) from a logistic GLMM of fixed covariates Time post-acquisition, Season and their interaction on the proportion of infected *Philaenus spumarius* and the proportion of infected olive seedlings in kinetic experiment.

	PCR-positive insects	Plants infected
Time post-AAP	1.01	0.99
	(1.00–1.02)	(0.97–1.02)
Season	4.95 ***	0.93
	(2.84–8.62)	(0.20–4.34)
Time post-AAP x Season	0.95 **	1.03
	(0.92–0.99)	(0.96–1.12)
N	538	82
N (Year)	2	2
SD (Year)	0.63	0
BIC	720.49	126.79
R2 (fixed)	0.08	0.03
R2 (total)	0.18	0.03

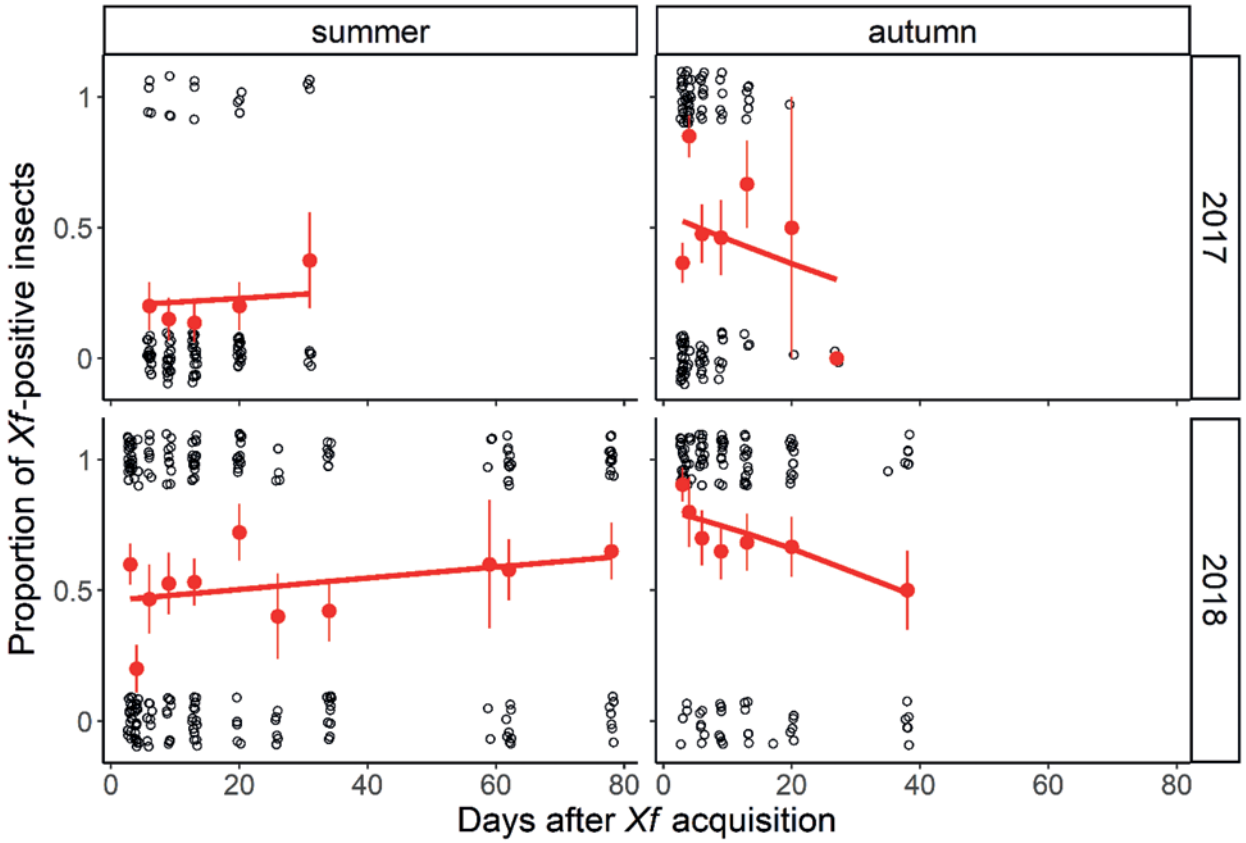

Fig. 1. Proportion of *Xf*-positive *Philaenus spumarius* individuals at different time points after acquisition from infected olive branches during summer and autumn 2017-2018 assays. Hollow points represent the results of single insects (positive = 1, negative = 0); a small amount of variation is included to make visible the number of insects tested at each time point even if the multiple points had the exact same value. Red filled points and line represent the proportion of positive insects (mean ± SE) and the red lines the outcome of binomial GLMM model; data from insects collected immediately after AAP end were excluded from depicted GLMM model, to avoid biased estimation of infected insects.

between the 13th and 20th post-AAP days (median: 3,154 cells/insect) (Fig. 2). The growth of *Xf* population in insects was explained slightly better by the Michaelis-Menten model than by the linear model in the summer assays and in the autumn 2018 assay (summer 2017: AIC_{linear} = 79.5, $AIC_{M\text{-}M}$ = 79.4; summer 2018: AIC_{linear} = 399.3, $AIC_{M\text{-}M}$ = 393.0; autumn 2018: AIC_{linear} = 358.4, $AIC_{M\text{-}M}$ = 356.6), while the growth of the *Xf* population was described better by the linear model in the autumn 2017 assay, probably because the assay ended earlier than in 2018, and the *Xf* population did not reach a plateau (AIC_{linear} = 260.2, $AIC_{M\text{-}M}$ = 262.9). In 2018 assays, the estimated asymptote of mean number of *Xf* cells in the foregut of infected *P. spumarius* was different between the summer assays (2,455 cells, CI = 441–2,698) and autumn ones (4,335 cells, CI = 1,729–12,543), with a plateau being reached around 20 days after acquisition (Fig. 2). However, in summer 2018, there were PCR-positive insects with a low number of *Xf* cells, i.e. 20–50, even at 60–78 days post-AAP.

3.3 *Xf* transmission to olive plants

Following acquisition from field-grown *Xf*-infected olive trees, *P. spumarius* adults were transferred to olive seedlings for a 3-day IAP at different post-acquisition times (up to 78 days post-AAP in summer 2018 assay). Overall, 29 out of 82 olive seedlings were positive for *Xf* (35.4%) twelve months after inoculation. Percentage of *Xf*-positive olive seedlings 12 months after inoculation, i.e. a proxy of inoculation success of *Xf* by *P. spumarius*, was variable among the assays and the post-AAP times, e.g. the IQR percentage of infected olive seedlings was between 21% and 57% of tested plants (Fig. 3). The proportion of infected PCR-tested olive seedlings was not affected significantly by either *Time post-AAP* (χ^2_1 = 0.33, P = 0.856), *Season* (χ^2_1 = 0.77, P = 0.378) or their interaction (χ^2_1 = 0.69, P = 0.403) (Tab. 1). In other words, inoculation rate was constant – and randomly variable – across samples, with an average inoculation rate of 34%. The likelihood of *Xf* transmission to olives increased significantly as the average *Xf* population in insects augmented,

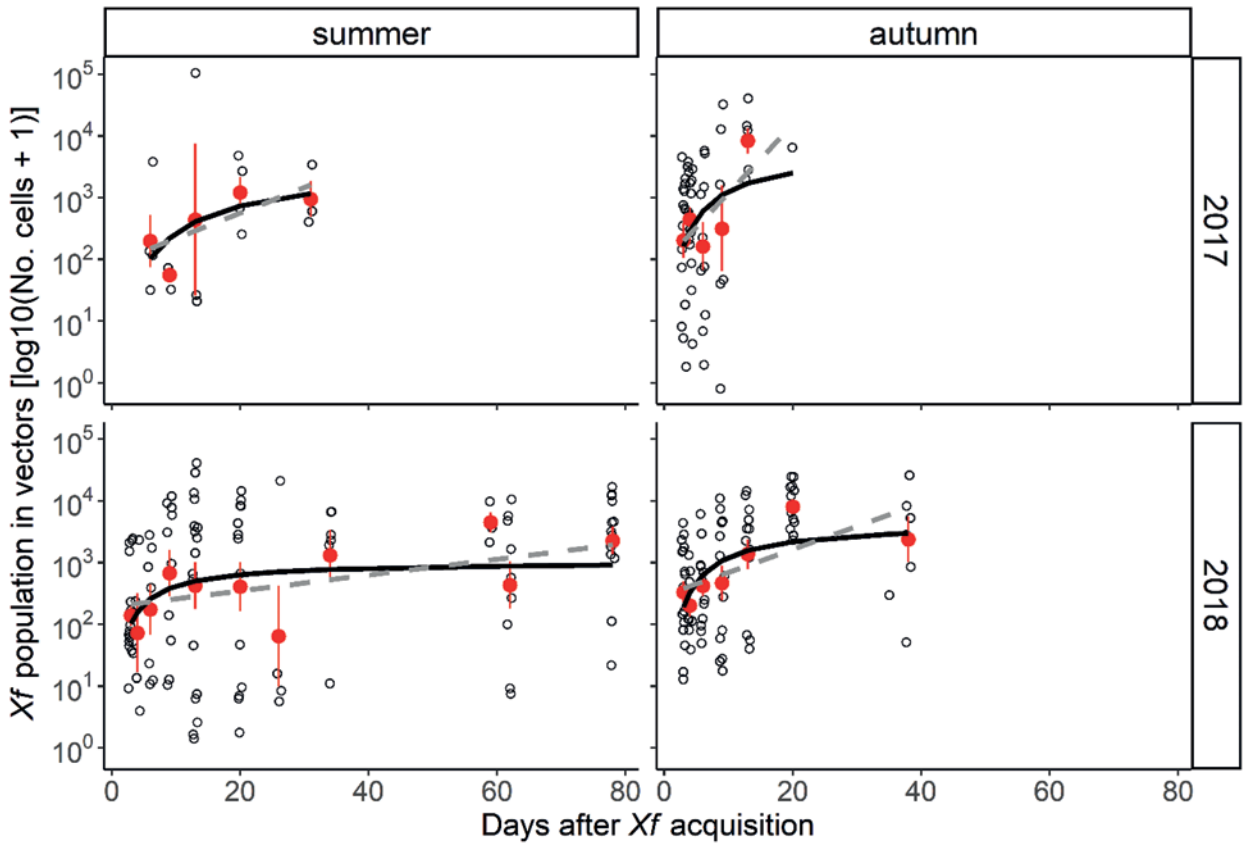

Fig. 2. qPCR-estimated population of Xf in the foregut of Philaenus spumarius (decimal log-transformed no. of cells) at different time points after acquisition from infected olive branches during summer and autumn 2017-2018 assays. Hollow points represent the results of single positive insects, red filled points represent the proportion of positive insects (mean + SE), continuous black lines indicate the outcome of Michaelis-Menten model and the dashed grey lines show the outcome of linear model.

regardless of the season (*Xf population*: χ^2_1 = 5.72, P = 0.017; *Season*: χ^2_1 = 0.50, P = 0.479) (Fig. 4; Supplementary Tab. 2). Conversely, the number of PCR-positive insects did not significantly affect the likelihood of *Xf* transmission to the olives, i.e. the olive seedlings inoculated with a higher number of *Xf*-positive insects (range: 1-4 positive insects/batch) were not significantly more likely to become infected than those inoculated with fewer infected insects (*Number of Xf-infected insects*: χ^2_1 = 1.05, P = 0.306) (Supplementary Tab. 2). Although not statistically significant, the relationship between the number of positive insects and the successful transmission showed an apparent trend, as a recipient olive seedling exposed to four *Xf*-positive *P. spumarius* is on average 2.6-fold more likely to be infected than one exposed to a batch containing a single positive insect.

3.4 Inoculation rate estimates

The Poisson model provided a better fit to the kinetic transmission dataset than the binomial model, based on AIC score. The overall inoculation rate of PCR-positive vectors – b (proportion/vector/day) – as estimated by the Poisson model, was 0.062 ± 0.014 (t_{16} = 4.44, P < 0.001), when all the assays performed during the different seasons were pooled. When the assays performed in summer and autumn were considered separately, they provided different estimates, i.e. lower in summer (0.034 ± 0.015, t_8 = 2.24, P = 0.05) than in autumn (0.08 ± 0.02, t_7 = 4.06, P = 0.005).

3.5 Spread of *Xf* by *P. spumarius* under microcosm conditions

Overall, the recapture rate of live *P. spumarius* from microcosms at the end of IAPs, i.e. survival rate, was 48% (1,127 alive out of 2,352 insects released). The survival of insects in microcosms was affected by both the interaction of IAP duration and season (*IAP duration* × *Season*: χ^2_1 = 26.87, P < 0.001), and the interaction of season and climatic conditions (*Season* × *Climatic condition*: χ^2_1 = 6.43, P = 0.011) (Tab. 2). Therefore, a lower survival rate was recorded at the end of longer IAPs, especially during the autumn assays, e.g. at the end of 21-day long IAPs, only 3% (2017) and 4% (2018)

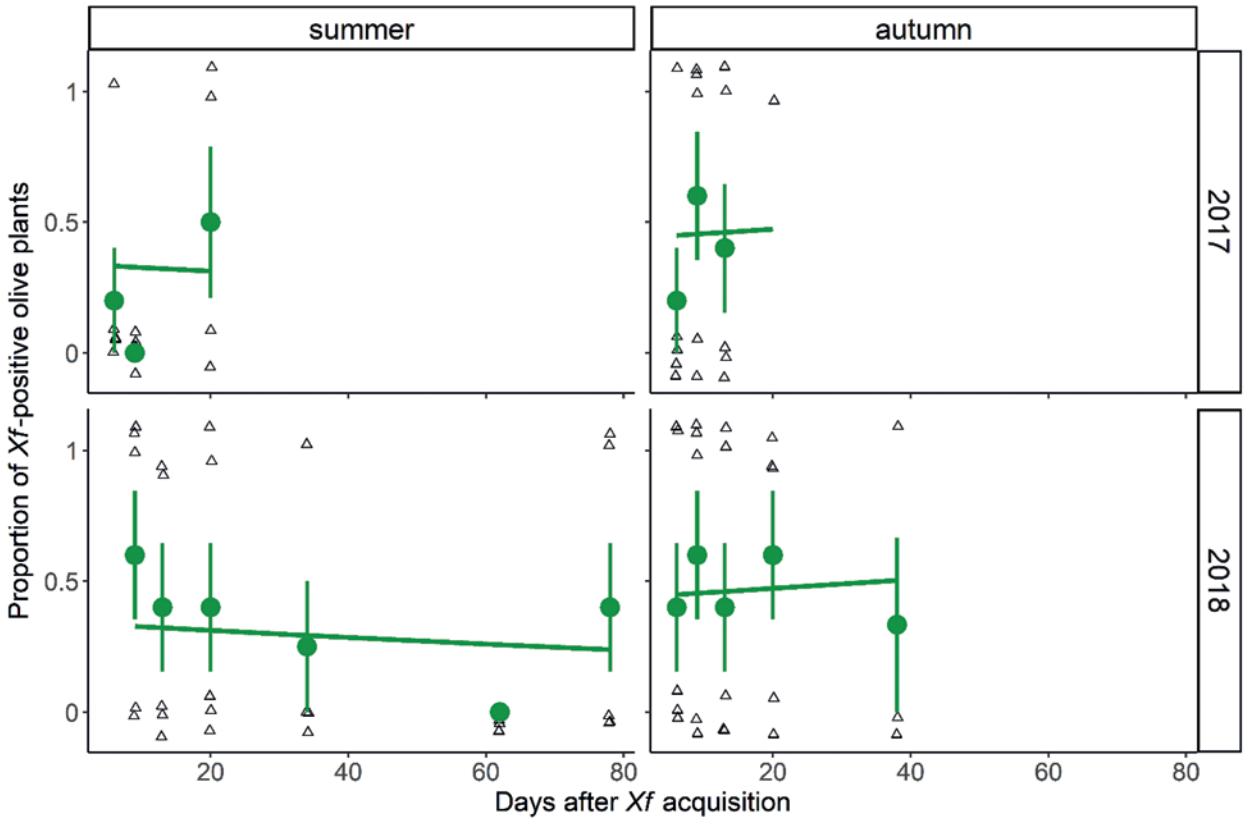

Fig. 3. Proportion of *Xf*-positive olive seedlings inoculated by batches (n = 5) of *Philaenus spumarius* at different time points after acquisition from infected olive branches during summer and autumn 2017-2018 assays. Hollow triangles represent the results of single plants (positive = 1, negative = 0); a small amount of variation was included to make visible the number of plants tested at each time point, even if the multiple points had the exact same value. Green filled points represent the proportion of positive olive plantlets (mean ± SE) and the green lines represent the outcome of binomial GLM model described in Table 1.

of the insects released inside the microcosms were collected alive, irrespective of the climatic conditions. Survival rate of caged *P. spumarius* was higher in summer, especially under controlled climatic conditions (2017: 66.7%, 2018: 85.2%), than in semi-field conditions (2017: 28.9%, 2018: 49.2%).

The effects of season, days after AAP, and climatic conditions on the proportion of infected *P. spumarius* were only analysed for the 2018 assays, given the low number of infected insects recollected during the 2017 assays (n = 32, 3.9%). In 2018 assays, there was a significant interaction between the three covariates – *IAP duration, Season*, and *Climatic condition* – on the proportion of infected recaptured insects ($\chi^2_1 = 8.78$, P = 0.003) (Tab. 2). In other words, the proportion of infected *P. spumarius* was initially lower in summer (IQR: 31-35%) than in autumn (IQR: 66-77%), and then, during summer, it decreased slightly over time under both the controlled and semi-field conditions, while the proportion of infected *P. spumarius* abruptly decreased in autumn for longer IAPs under semi-field conditions, but remained quite stable under controlled climatic conditions (Fig. 5).

Similarly, the *Xf* population in PCR-positive *P. spumarius* was only analysed for the 2018 assays (n = 444). The PCR-positive individuals on average had higher *Xf* populations after longer IAPs (that is, a longer post-AAP period) (*IAP duration*: $F_{1,375} = 8.75$, P = 0.003). Moreover, *P. spumarius* in the autumn assays had higher mean *Xf* loads than those tested in summer (*Season*: $F_{1,375} = 35.49$, P < 0.001) (Supplementary Tab. 3). Considering the total number of recollected insects (both PCR-positive and -negative individuals) in the 2018 assays (n = 957), the mean *Xf* population was higher in autumn, as expected by the higher rate of positive insects (*Season*: $F_{1,696} = 10.13$, P = 0.001) (Tab. 2). However, a significant interaction was observed between *Season, Climatic condition*, and *IAP duration* ($F_{1,696} = 8.38$, P = 0.004), that is, the average *Xf* population in the insects collected alive at the end of IAP in autumn was high under both conditions for shorter IAPs, and then gradually declined under semi-field conditions, while it remained stable under controlled conditions; on the other hand, the mean *Xf* population gradually declined under both conditions in the summer assays (Fig. 6).

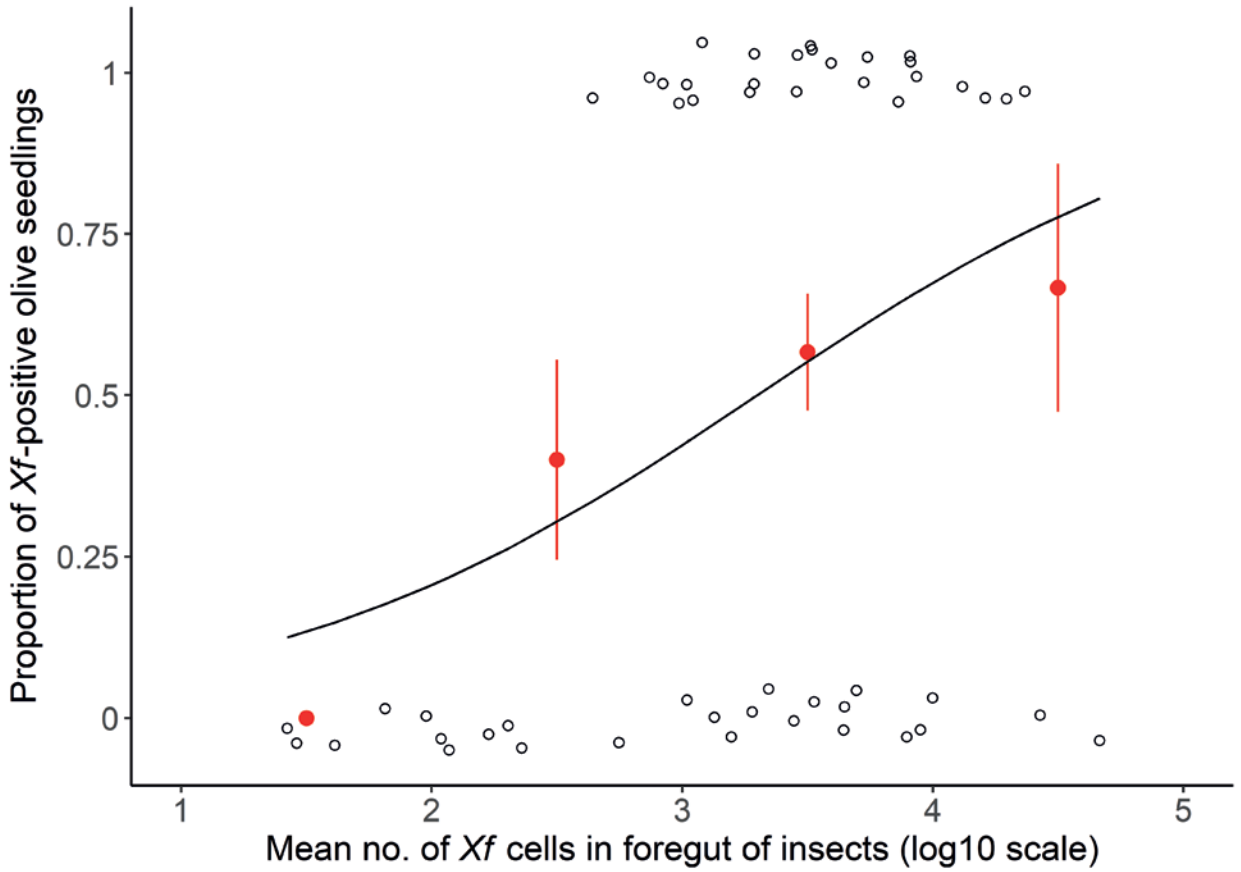

Fig. 4. Proportion of *Xf*-positive olive plantlets related to the mean *Xf* population size (decimal log of no. of cells) in *Philaenus spumarius* inoculation batches. Hollow points represent the result of single plants (positive = 1, negative = 0); a small amount of variation was included to make visible the number of tested plants even if the multiple points had the exact same value. Red filled points represent the proportion of *Xf*-positive olive plantlets for each binned log span of the mean *Xf* population size in a *P. spumarius* inoculation batch. The red line represents the outcome of binomial GLMM model.

3.6 Trasmission to olive seedlings

The transmission rate, i.e. the proportion of infected olive seedlings, was affected by Climatic condition (χ^2_1 = 10, P = 0.001) and Year (χ^2_1 = 23.3, P < 0.001), with significant interactions between Climatic condition × Year (χ^2_1 = 6.5, P = 0.011) and IAP duration × Season (χ^2_1 = 7.17, P = 0.007) (Tab. 2). The proportion of infected olive seedlings was higher after longer IAPs in autumn, and higher under controlled conditions than under semi-field conditions, especially in 2017 trials (2017: 10-fold higher likelihood of becoming infected than plants under semi-field conditions, 2018: 1.5-fold). Given the higher number of insects released in microcosms, the infection rates were higher in the 2018 assays than in the 2017 ones (plants had a 34-fold higher likelihood of becoming infected in 2018) (Fig. 7 and Tab. 2). In other words, the spread of infection among olives only increased with the inoculation time in late season, especially under controlled climatic conditions and with a higher vector density.

4 Discussion

We studied various aspects of the biology of *Xf* transmission by *P. spumarius* on olive plants in order to shed light on the epidemiology of this invasive bacterium in Europe and on the associated emerging diseases. Our results suggest that i) *P. spumarius* is a competent vector of *Xf* on olive throughout its adult life, ii) the *Xf* acquisition rate of the vector varies between seasons under field conditions, iii) the bacterium load in the foregut of the vector increases during the first 2-3 weeks after acquisition, and then becomes stable, iv) the transmission rate to olive seedlings remains constant at different post acquisition times, but tends to increase for longer inoculation times (although differently between seasons and under different climatic conditions), v) insect survival is influenced by the season (possibly a proxy of the insect's age), climatic conditions and IAP duration, affecting the transmission outcome of microcosm experiments, and possibly influencing the spreading rate of *Xf* in olive groves.

Table 2. Parameters estimated from logistic GLMM or linear regression of fixed covariates Inoculation duration, Climatic condition, Season and Year on the proportion of infected *Philaenus spumarius*, *Xylella fastidiosa* population size at the end of IAP in collected individuals and the proportion of infected olive seedlings in spread rate experiment.

	Survival rate	**PCR-positive insects[a]**	***Xf* population (pos + neg)[b]**	**Plants infected[a]**
IAP duration	0.93 ***	0.97	-0.13 ***	1.02
	(0.91–0.95)	(0.92–1.01)	(-0.20–-0.06)	(0.97–1.06)
Climatic conditions	5.99 ***	1.38	0.60	10.89 **
	(3.74–9.58)	(0.69–2.77)	(-0.59–1.79)	(2.48–47.80)
Season	1.94 **	8.09 ***	1.84 **	0.57
	(1.31–2.89)	(3.43–19.07)	(0.70–2.98)	(0.25–1.30)
Year	–	–	–	34.54 ***
				(8.20–145.47)
IAP duration × Climatic conditions	1.00	1.01	0	–
	(0.97–1.03)	(0.95–1.07)	(-0.09–0.08)	
IAP duration × Season	0.87 ***	0.86 **	-0.08	1.08 **
	(0.84–0.90)	(0.77–0.96)	(-0.20–0.03)	(1.02–1.15)
Season × Climatic conditions	0.29 ***	0.19 **	-1.47	–
	(0.19–0.43)	(0.06–0.56)	(-2.97–0.04)	
Climatic conditions × Year	–	–	–	0.13 *
				(0.03–0.63)
IAP duration × Climatic conditions x SeasonAutmn	–	1.21 **	0.23 **	–
		(1.07–1.38)	(0.07–0.38)	
N	96	829	704	1489
N (replica)	–	3	–	–
N (year)	2	–	–	96
BIC	530.96	1126.39	3545.95	928.86
R2 (fixed)	0.38	0.11	–	0.43
R2 (total)	0.42	0.11	0.18	0.45

[a] Logistic GLMM, estimated odds ratios, z-statistics significance and confidence interval (95%)
[b] Linear regression, estimated intercept and slope parameters, ***t***-statistics significance and confidence interval (95%)

Unexpectedly, the higher acquisition rate of *Xf* by *P. spumarius* in the autumn assays than in the summer ones did not correspond to an increase in *Xf* populations in the olive source plants, although the acquisition rate of *Xf* by xylem-sap feeders is usually related to the bacterium population in the source plant (Hill & Purcell 1997). In our experiments, the estimated *Xf* loads in the source plants were slightly higher in summer than in autumn, contrary to the expectation that *Xf* population in plant tends to increase during the vegetative season (Hill & Purcell 1997; Giampetruzzi et al. 2020).This discrepancy may be due to the sample collection of the leaf tissues, which in summer included mature leaves produced on the shoots of the previous year, and thus possibly harbouring a higher number of *Xf* cells – dead or alive – than in the late season samples, whereas sampling in autumn included mature leaves from shoots grown during the same year and thus less heavily colonised by *Xf*. Alternatively, the limited number of leaves/branch used in our tests may help explain the results, given the heterogeneous distribution of *Xf* in the infected plants.

Once acquired, *Xf* multiplied in the foregut of *P. spumarius,* reaching an average of a few thousand cells after 2–3 weeks, although the *Xf* population was estimated to exceed forty thousand cells in few individuals (n = 3). The average number of cells of the *Xf* population in the mouthparts of the spittlebugs at plateau was higher in the autumn assays than in the summer ones. The maximum number of *Xf* cells in the foregut of *P. spumarius* was higher than the number estimated in previous studies, possibly because *Xf* population in the vector was tested i) only a few days after post-acquisition (Cavalieri et al. 2019), or ii) in field-collected individuals, whose acquisition conditions were unknown (Cornara et al. 2017a), or iii) in spittlebugs exposed to different *Xf* subspecies/host plant combinations (e.g. *Xf*. subsp. *fastidiosa* in grapevine) (Cornara et al. 2016). Indeed, our estimates of *Xf* population in the vector eight days after the start of

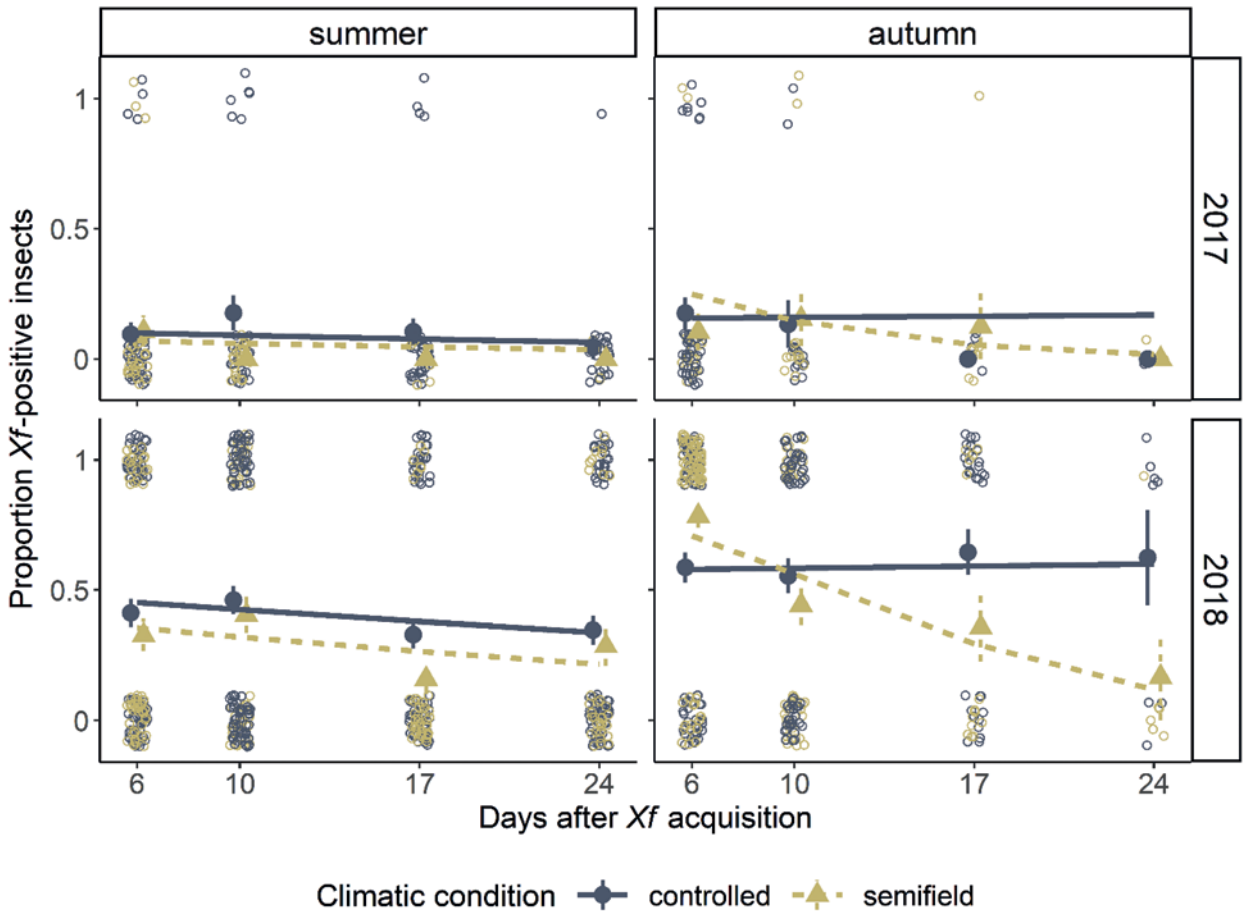

Fig. 5. Proportion of *Xf*-positive *Philaenus spumarius* individuals after different inoculation periods in *Xf* spread experiments during summer and autumn 2017-2018 assays. Note that x-axis scale is equal to IAP duration + 3 days of AAP. Hollow points represent the result of single insects (positive = 1, negative = 0); a small amount of variation was included to make visible the number of insects tested at each time point even if the multiple points had the exact same value. Filled points and triangles represent the proportion of positive insects (mean ± SE) recollected at different IAPs, while the lines represent the outcome of binomial GLMM model for different climatic conditions. A small amount of horizontal variation was included for points and triangles to avoid overlapping.

AAP were similar to those Cavalieri et al. (2019) tested at approximately the same time after the beginning of acquisition, i.e. 400–900 cells. Moreover, the maximum population size measured in the head of *P. spumarius* in our work is compatible with the number of *Xf* cells potentially hosted by *P. spumarius,* as estimated on the basis of the cuticular surface available in the foregut (≈ 67,000 cells) (Ranieri et al. 2020). Thus, our results show that *P. spumarius* may harbour similar *Xf* loads in its foregut to the ones measured in the main sharpshooter vectors in american *Xf* subsp. *fastidiosa*-grapevine pathosystem, e.g. *G. atropunctata* and *H. vitripennis* (Hill & Purcell 1995; Almeida & Purcell 2003; Killiny & Almeida 2009), although the average bacterial load is usually higher in sharpshooters foregut.

Significant decreases were observed in the proportion of infected insects, in the mean *Xf* load in insects alive at the end of IAP, and in the survival rate during the autumn assays. Such a trend could be explained by a higher mortality of *Xf*-infected insects than of healthy ones, especially under the stressing conditions of cages under the semi-field conditions in autumn (colder climate and higher daily temperature variations), when insects are old and approaching the end of their life. This hypothesis would be consistent with the possible negative impact of *Xf* on the fitness and survival of the spittlebugs, caused by the precibarium bacterial colonisation and the consequent reduction of food canal lumen. This reduction might lead to an additional energy requirement to ingest the xylem sap (Ranieri et al. 2020), and to variations in the feeding behaviour (e.g. shorter xylem ingestion time and longer non-probing periods) (Cornara et al. 2020).

The transmission efficiency in the kinetics of *Xf* colonisation experiment, i.e. the likelihood of a successful inoculation of *Xf* to olive, increased with the size of the bacterium population in vectors, while it was not significantly affected

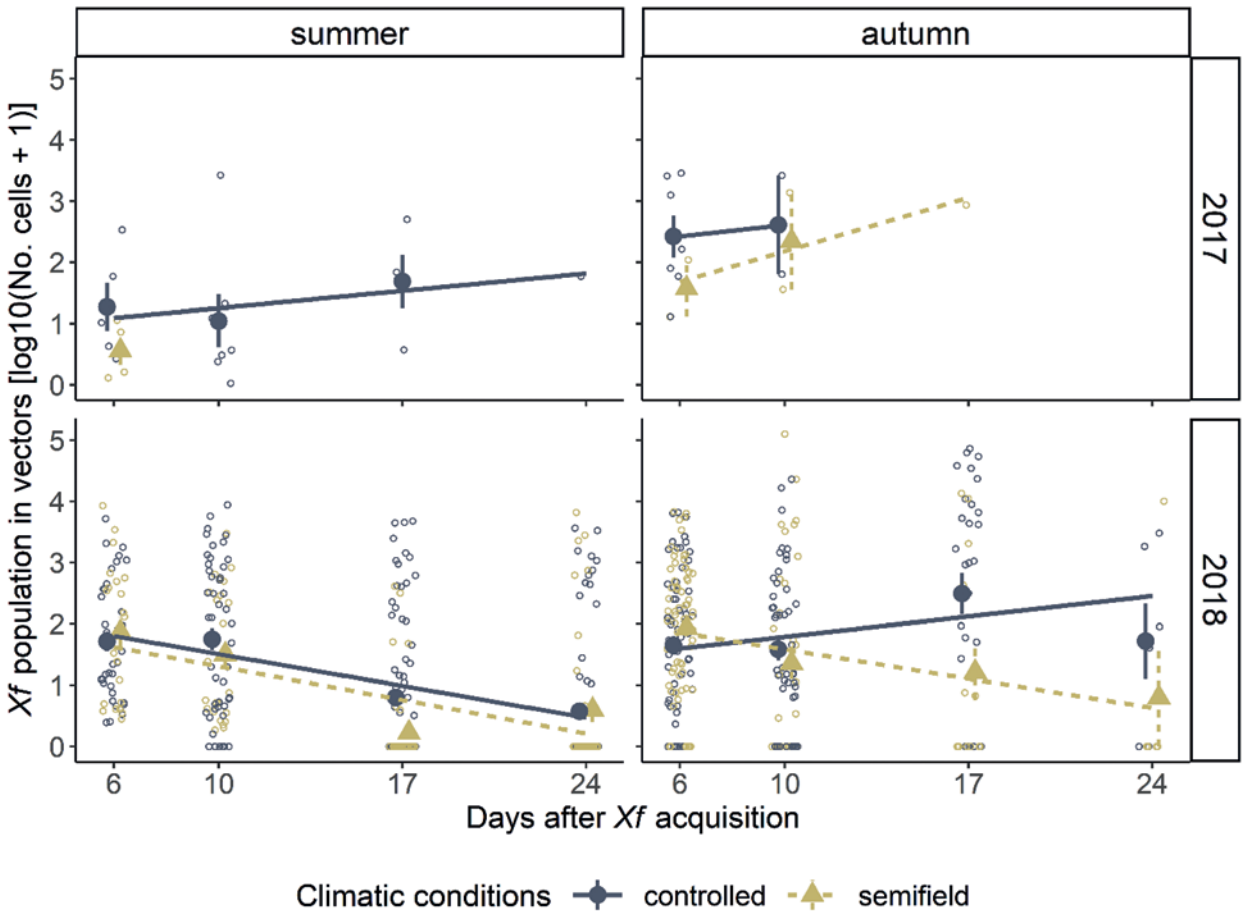

Fig. 6. qPCR-estimated population of Xf in the foregut of Philaenus spumarius (decimal log-transformed no. of cells) of individuals recollected alive at the end of IAPs in Xf spread experiments during summer and autumn 2017-2018 assays. Note that x-axis scale is equal to IAP duration + 3 days of AAP. Hollow points represent the results of single insects. Filled points and triangles represent the mean (±SE) number of Xf cells in the recollected P. spumarius at different IAPs, while the lines indicate the outcome of linear regression model for different climatic conditions.

by the number of infected insects per inoculation batch, the seasonal dynamics of acquisition, or the post-acquisition time during the kinetic experiment. The transmission efficiency of a batch composed of five *P. spumarius* was on average 34%, without any significant changes or trends over post-acquisition time or between seasons. In other words, small groups of vectors on a single olive plant and under controlled conditions inoculate at a constant rate, irrespective of post-acquisition time and season. However, the increase in the average *Xf* population per insect of one order of magnitude (e.g. from 10^2 to 10^3 cells) led to a 3.2-fold increase in the likelihood of olive infection. Moreover, the transmission efficiency did not increase for longer post-acquisition times (i.e. a longer *Xf* incubation time in the foregut of the vector), likely because the bacterial population reached a plateau. A positive correlation has already been observed between the transmission rate and bacterial load in vectors for the transmission of *X. fastidiosa* subsp. *fastidiosa* by *P. spumarius* and *G. atropunctata* from grapevine to grapevine (Cornara et al. 2016; Zeilinger et al. 2018).

The overall estimated average inoculation rate – after 3 days of AAP – was 6% of infected plants per insect per day. When considering the results obtained in different seasons, the inoculation rate per insect was higher in autumn than in summer. In the literature on *X. fastidiosa* subsp. *fastidiosa*, higher inoculation rate estimates have been reported for *P. spumarius* (≈ 15 %) and *G. atropunctata* (≈ 20 %) on grapevine (Cornara et al. 2016; Daugherty and Almeida 2009), while a similar rate has been reported for *Draeculacephala minerva* Ball on periwinkle from almond (in average 9%) (Cabrera-La Rosa et al. 2008), and usually lower rates have been reported for *H. vitripennis* on grapevine and almond (2.5 – 4.5 % infected plants/insect/day) (Almeida and Purcell 2003; Daugherty & Almeida 2009). According to Marucci

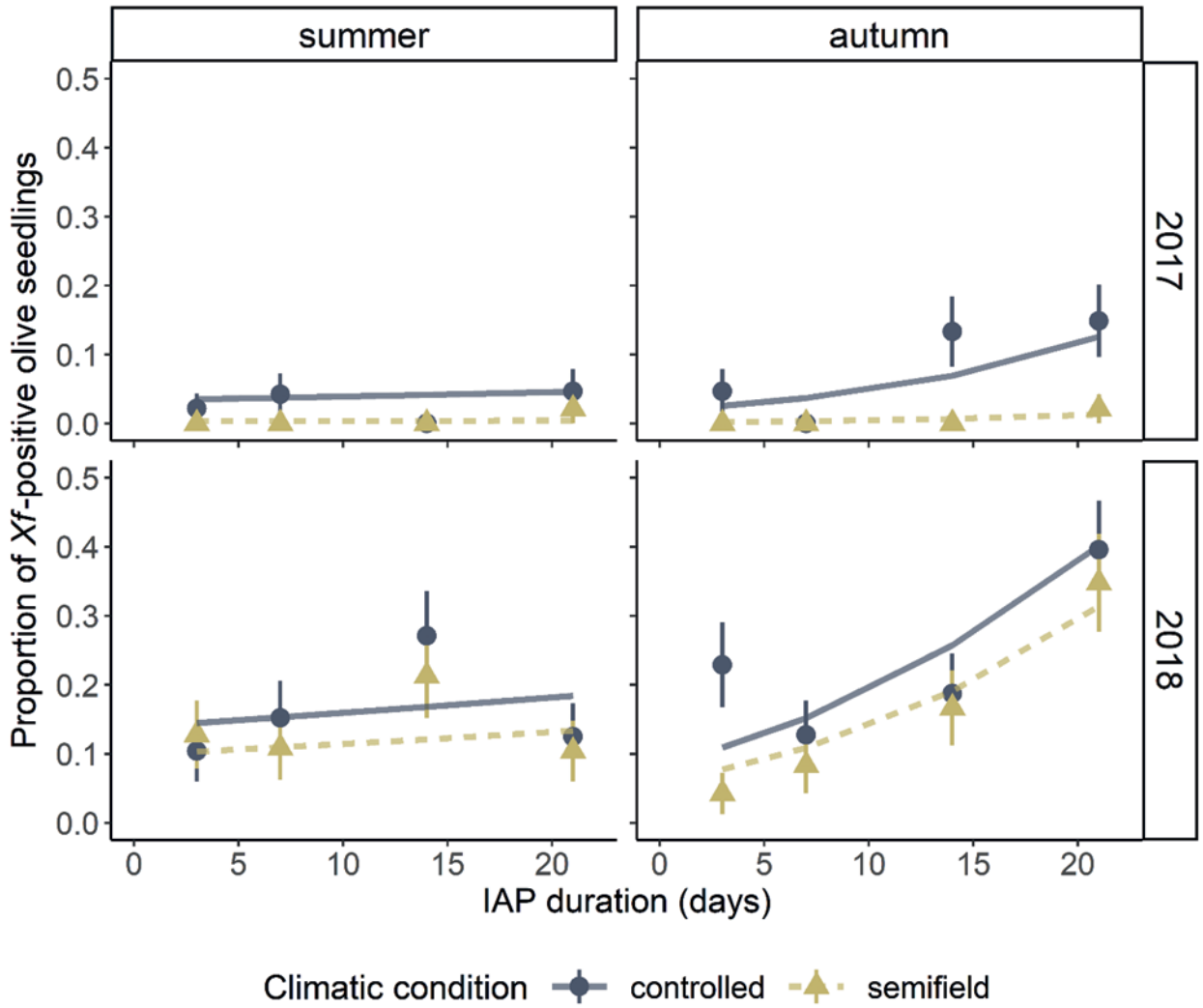

Fig. 7. Proportion of infected olive seedling after different inoculation periods in *Xf* spread experiments during summer and autumn 2017-2018 assays. Points and triangles represent the proportion of positive *P. spumarius* (mean + SE), while the continuous and dashed lines indicate the outcomes of binomial GLMM model for different climatic conditions (points-continuous lines = controlled; triangles-dashed lines = natural).

et al. (2008) and Lopes & Krugner (2016), the inoculation efficiency of CVC strains of *X. fastidiosa* subsp. *pauca* on citrus of the main vector species [e.g. *Dilobopterus costalimai* Young, *Acrogonia citrina* Marucci & Cavichioli, *Oncometopia facialis* (Signoret) and *Bucephalogonia xanthophis* (Berg)] was lower than or similar (1–13% over a two-day IAP) to that estimated for *P. spumarius* in this study. The inoculation rate of *P. spumarius* on olive calculated on data from Cavalieri et al. (2019), that is, 6–7% over a four-day IAP, as obtained with the Swallow formula (Swallow 1985), is lower than our estimate of 6% per day. However, the methodological differences between the different studies – e.g. AAP and IAP durations, source plants, periods of the year, statistical approaches adopted – suggest caution when comparing the outcomes of different studies. Moreover, in some studies, e.g. Daugherty & Almeida (2009), estimates were obtained also by reviewing datasets from several studies, whose methodologies were not homogeneous. It is worth pointing out that our estimates were taken from an experiment that had not been specifically intended to explore the effects of inoculation time and number of insect vectors per plant, as those parameters were fixed (3 days and 5 insects respectively), and thus can be considered reliable for those conditions, although the inoculation rate could vary for different combinations of inoculation duration and number of inoculative insects (e.g. Daugherty & Almeida 2009; Cornara et al. 2016).

In the present study, the spreading rate of the *Xf* epidemic on olive plants by *P. spumarius*, i.e. the increase rate of the proportion of infected plants with the inoculation time within an olive population, was affected by different factors, such as the duration of IAP, the season and the climatic conditions. Indeed, the spreading rate of *Xf* was higher under controlled vs semi-field climatic conditions in all the assays, which could mean that more controlled climatic conditions – e.g. more stable compared to the semi-field conditions, with lower temperatures in the summer assays and higher in the autumn ones, or lower daily variations – resulted in a faster spread of *Xf* among olive seedlings, possibly due to higher inoculation rates and/or higher mobility and survival of *P. spumarius* in the microcosms. Furthermore, the spreading rate of *Xf* on olives increased with inoculation time only in the autumn assays, irrespective of climatic conditions. Although the transmission rate is expected to increase for longer inoculation periods (Purcell & Finlay 1979; Daugherty & Almeida 2009; Cornara et al. 2016), the behaviour of the vectors – e.g. their preference for low water stressed plants – may also influence the visiting of host plants and, ultimately, the transmission rate (Mizell et al. 2008; Del Cid et al. 2018). In our case, the observed seasonal variation of *Xf* spreading rate on olive seedlings could be partially explained by a higher mobility of the spittlebug in autumn, given that *P. spumarius* tends to move to herbaceous species late in the season for mating and oviposition (Bodino et al. 2020). The vector movement propensity may be higher in this period, especially in a microcosm environment that does not provide good oviposition sites, thus possibly forcing the vector to visit more olive seedlings. Finally, the *Xf* spreading rate on olives was significantly higher in the 2018 assays, when the vector density was about the double.

In summary, this work describes the influence of seasonal and environmental factors on the transmission characteristics of *Xf* by *P. spumarius*. Such transmission dynamics could contribute significantly to determining the epidemic patterns in the Apulian olive agroecosystem. The highest acquisition and overall transmission rates of *Xf* in the Apulian olive agroecosystem probably occur in early summer, as this is the period with the highest density of vectors on olive canopies (Cornara et al. 2017b; Bodino et al. 2019, 2020). However, because *P. spumarius* is infectious throughout its adult life, our results indicate that the transmission/spreading efficiency may even increase in the late season. Thus, also the role of the few spittlebugs visiting olive canopies late in the season could be epidemiologically relevant. Moreover, the inoculation events that take place late in the season could contribute to the spread of *Xf* to greater distances from the source plants, as the distance travelled by an insect is a function of time, being in the 350-650 m range after 5–6 months from adult emergence in olive groves (Bodino et al. 2021). Thus, older *P. spumarius* adults could play a role in dispersing the bacterium at longer distances onto olive trees or wild plants within the agroecosystem, thereby expanding the infected area. Further studies on the spread of *Xf* under field or semi-field conditions, as well as on seasonal variations of the dispersal and mobility of vectors are needed to gain more knowledge on the factors that drive the epidemic of *Xf* on olive trees. Besides transmission efficiency, *P. spumarius* population level and preference for olive plants are major drivers of *Xf* spread in olive groves. It is crucial to reduce the *P. spumarius* populations in the olive groves in Apulia, since the number of feeding insects is probably the most important factor in determining the successful transmission and quick spread of the pathogen (Daugherty & Almeida 2009).

In conclusion, the transmission parameter estimates reported here, and their variation over time, should be used to inform the epidemic spread models of *Xf* in Italy and Europe that explicitly account for the effect of vectors. *Philaenus spumarius* and its interactions with *Xf* are crucial factors that affect the establishment, persistence and epidemic spreading of the exotic pathogen.

Authors' Contributions: conceptualisation, N.B., V.C., R.P.P.A., C.D., M.S., D.B.; methodology, N.B., V.C., C.D., M.P., M.S., D.B; data curation, N.B.; statistical analysis, N.B; investigation, N.B., V.C., C.D., F.C., M.P., G.A., S.Z., G.F.; resources, M.S., D.B.; writing–original draft preparation, N.B.; writing–review and editing, N.B., V.C., C.D., F.C., M.S., R.P.P.A., D.B.; supervision, D.B.; project administration, M.S., D.B.; funding acquisition, M.S., D.B.

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Appendix

Supplementary Table 1. Assays outline and summary of kinetic experiment.

Year	Season	days after start AAP	Inoculation plant	No. replicates	*Xf*-positive insects (Proportion)	No. insects inoculated on olive	No. insects tested
2017	Summer	6	Olive	5	0.2	25	20
2017	Summer	9	Olive	5	0.15	25	20
2017	Summer	13	Olive	5	0.136	25	22
2017	Summer	20	Olive	5	0.2	25	20
2017	Summer	31	No_inoculation	1	0.375	0	8
2017	Autumn	3	No_inoculation	1	0.366	0	41
2017	Autumn	4	No_inoculation	1	0.85	0	20
2017	Autumn	6	Olive	5	0.476	25	21
2017	Autumn	9	Olive	5	0.462	25	13
2017	Autumn	13	Olive	5	0.667	25	9
2017	Autumn	20	Olive	1	0.5	5	2
2017	Autumn	27	No_inoculation	1	0	0	3
2018	Summer	2	No_inoculation	1	0.9	0	20
2018	Summer	3	No_inoculation	1	0.2	0	20
2018	Summer	5	Olive	6	0.467	30	15

Supplementary Table 1. continued.

Year	Season	days after start AAP	Inoculation plant	No. replicates	*Xf*-positive insects (Proportion)	No. insects inoculated on olive	No. insects tested
2018	Summer	8	Olive	5	0.526	25	19
2018	Summer	12	No_inoculation	1	0.6	0	10
2018	Summer	12	Olive	5	0.5	25	22
2018	Summer	19	Olive	5	0.722	25	18
2018	Summer	25	No_inoculation	1	0.4	0	10
2018	Summer	33	Olive	5	0.368	25	19
2018	Summer	58	No_inoculation	1	0.5	0	4
2018	Summer	61	No_inoculation	1	1	0	1
2018	Summer	61	Olive	5	0.579	25	19
2018	Summer	77	Olive	5	0.65	25	20
2018	Autumn	3	Olive	1	0.905	0	21
2018	Autumn	6	Olive	5	0.7	25	20
2018	Autumn	7	Olive	1	0.8	0	10
2018	Autumn	9	Olive	5	0.65	25	20
2018	Autumn	13	Olive	5	0.684	25	19
2018	Autumn	17	Olive	1	0	0	1
2018	Autumn	20	Olive	5	0.667	25	18
2018	Autumn	35	Olive	1	1	0	1
2018	Autumn	38	Olive	3	0.5	15	12

Supplementary Table 2. Estimated parameters from logistic GLM of fixed covariates Mean *Xylella fastidiosa* population size in vector batch, Number of *Xf*-positive individuals in inoculation batch on proportion of infected olive seedlings in kinetic experiment.

	Proportion of *Xf*-positive plants
Xf population size (log10)	2.65 *
	(1.11–6.35)
No. *Xf*-positive insects in batch	1.38
	(0.74–2.59)
Season	0.64
	(0.19–2.17)
N	51
AIC	70.05
BIC	77.77
Pseudo R^2	0.21

Supplementary Table 3. Estimated parameters from logistic GLMM or linear regression of fixed covariates Inoculation duration, Climatic condition and Season on *Xylella fastidiosa* population size in qPCR-positive individuals only in spreading rate experiment.

	***Xf* population (positives only)**
IAP duration	0.06 **
	(0.02–0.10)
Climatic conditions	0.44
	(-0.01–0.89)
Season	1.44 ***
	(0.97–1.91)
N	383
N (Year)	–
AIC	1680.43
BIC	1700.17
R^2 (fixed)	–
R^2 (total)	0.10