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ORIGINAL PAPER

Transmission of *Xylella fastidiosa* subspecies *multiplex* from naturally infected to healthy *Rhamnus alaternus* by *Philaenus spumarius* and *Neophilaenus campestris*

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Abstract

In Europe, the recently reported plant pathogen *Xylella fastidiosa* subsp. *multiplex* affects several wild, ornamental, and cultivated trees causing scorch diseases. In 2018, the sequence type 87 was reported in Tuscany on Mediterranean shrubs and trees. Although spittlebugs (Hemiptera: Aphrophoridae) were already identified as main vectors of this bacterium in Europe, their role in the transmission of this subspecies has not been ascertained yet. In this study the ability of *Philaenus spumarius* and *Neophilaenus campestris* to acquire and transmit *Xylella fastidiosa* subsp. *multiplex* sequence type 87 from and to *Rhamnus alaternus* was evaluated in two-year semi-field experiments. To acquire the bacterium, insects were confined on wild, naturally infected *R. alaternus* shrubs for 120 h. Then, they were transferred to healthy plants and maintained in cages for 96 h. To follow the infection, plant samples were collected every two months for three times. Tested plants were destroyed at the end of experiments and roots, twigs and leaves were analysed. *Philaenus spumarius* showed a significantly higher survival rate than *N. campestris*. The infection status of both insects and plants was assessed through molecular analysis. *P. spumarius* and *N. campestris* were able to infect healthy plants although the acquisition rate and the estimated probability of transmission appeared to be low. These findings provide new accounts on the role of two polyphagous insect vectors in spreading a quarantine organism, which is lethal to a huge number of plant species. However, further studies are needed to disclose more specific interactions within this complex pathosystem.

Keywords Italian buckthorn · Mediterranean vegetation · Quarantine bacterium · Spittlebug · Vector

Key Message

- Spittlebugs are putative vectors of *Xylella fastidiosa* subspecies *multiplex* in Mediterranean countries.
- *P. spumarius* and *N. campestris*, positive to *X. fastidiosa multiplex*, were found on Mediterranean vegetation.
- Semi-field acquisition and transmission tests were performed using these two spittlebug species.
- *R. alaternus* was used as source and recipient plant in acquisition and transmission experiments.
- *P. spumarius* and *N. campestris* were able to infect healthy plants.

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Introduction

The plant-pathogen *Xylella fastidiosa* (Wells et al. 1987) is a Gram-negative bacterium, belonging to the family Xanthomonadaceae that colonizes the xylem of more than 600 plant species, including ornamental, landscape and cultivated herbs and trees (EFSA 2023). During its growth, the bacterium produces a biofilm and synthetizes several pathogenicity factors (Marques et al. 2002; Killiny et al. 2013; Rapicavoli et al. 2018). This metabolic activity can lead to the occlusion of the xylem vessels and to the development of scorch and dwarfing diseases in the infected plants (Janse and Obradovich 2010). In the Americas, *X. fastidiosa* severely affects grapevine causing the well-known Pierce's disease (PD) (Davis et al. 1978; Hopkins and Purcell 2002), as well as almond, peach, apricot, plum, pecan, blueberry, citrus, and coffee (EFSA 2018). Besides being pathogenic in more than 100 plant species (Rapicavoli et al. 2018), the bacterium can latently remain in hosts which do not show disease symptoms, representing a reservoir of *X. fastidiosa* in the environment (Chatterjee et al. 2008; Sicard et al. 2018).

Xylella fastidiosa's short range dispersion is mediated by insect vectors (Redak et al. 2004; Krugner et al. 2019), while its spread over longer distances is mostly due to the global plant trade (Sicard et al. 2018). Although all Hemipteran xylem sap feeders (Cicadoidea, Cercopoidea and Cicadellinae) could potentially acquire and transmit the bacterium, their actual role as vectors has been assessed only for some species, mainly belonging to the subfamily Cicadellinae and to the family Aphrophoridae (Redak et al. 2004; Cornara et al. 2017; Cavalieri et al. 2019; Krugner et al. 2019; Müller et al. 2021). On the contrary, cicadas (Hemiptera: Cicadidae) do not seem involved in the transmission of the bacterium, at least in Europe (Cornara et al. 2020; Mesmin et al. 2023).

Xylella fastidiosa is considered a genetically diverse species with three currently accepted subspecies named *fastidiosa*, *multiplex,* and *pauca* (Bull et al. 2012; Denancé et al. 2019), however, many strains have been described so far (Yuan et al. 2010; Giampetruzzi et al. 2015; Denancé et al. 2017; Saponari et al. 2019). Subspecies differ for the area of origin and for the host range, often showing a clear host-specificity also at strain level (Nunney et al. 2013). *Xylella fastidiosa* subsp. *fastidiosa* is native to southern Central America and is the causal agent of the Pierce's disease of grapevine, while *X. fastidiosa* subsp. *pauca* (XFP) originated in South America and infects mainly plants of the genera *Citrus* and *Coffea* (Almeida et al. 2008). However, in 2013 the sequence type (ST) 53 of XFP was reported for the first time in Europe and ascertained as the causal agent of the Olive quick decline syndrome (OQDS), a severe vascular disease that has led to the death of thousands of olive trees in Apulia, southern Italy (Saponari et al. 2013, 2017). After this first outbreak, XFP was also detected in France and in Balearic Islands (Denancé et al. 2017; Moralejo et al. 2019). Like XFP, *X. fastidiosa multiplex* (XFM) has recently spread in Europe, occurring in Central Italy (Tuscany and Latium), southern France, Corsica, Balearic Islands and mainland Spain (Alicante province), and Portugal (Denancé et al. 2017; Marchi et al. 2018; Trkulja et al. 2022; EPPO 2022; Cunty et al. 2022; Loureiro et al. 2023). XFM has the largest host range among the reported subspecies and is the only one native to the United States (Nunney et al. 2010). This subspecies is typically distributed in temperate zones and infects mainly tree species causing the Almond Leaf Scorch and other scorch diseases in wild and cultivated trees (Nunney et al. 2013). Almond trees are the most infected plants in Spain, while in France XFM primarily infects *Polygala myrtifolia* L. (Denancé et al. 2017; EFSA 2023). In Italy, XFM was reported in 2018 in the area of the Monte Argentario promontory in Tuscany (Marchi et al. 2018), where a different ST was identified and named ST87 (Saponari et al. 2019). The Tuscan outbreak is characterized by the infection of many Mediterranean landscape plants such as the Spanish broom (*Spartium junceum* L.*)*, the Italian buckthorn (*Rhamnus alaternus* L.) and the hairy thorny broom (*Cytisus laniger* DC.) (EFSA 2023; Fitosirt database, https://fitosirt.regione.toscana.it). This situation strongly resembles that of Corsica Island, where XFM prevails on XFP, infecting ornamental plants and wild species of the natural Mediterranean vegetation (Denancé et al. 2017; Cruaud et al. 2018; Cunty et al. 2022). Although XFM does not currently affect crops in Italy, it could threaten agricultural areas, since it could also infect many cultivated trees like the olive tree (EFSA, 2023). Moreover, its impact on the maquis, the native flora of the Mediterranean region, must not be overlooked.

As frequently underlined in previous studies, *X. fastidiosa* pathosystems may also be very different from one another, therefore overgeneralizing such acquired knowledge could lead to inaccurate conclusions (Sicard et al. 2018; Jeger and Bragard 2019; Desprez-Loustau et al. 2021). So far, spittlebugs in the family Aphrophoridae, especially *Philaenus spumarius* (Linnaeus 1758), were assessed to be the main European vectors of *X. fastidiosa* (Cornara et al. 2016, 2017; Cavalieri et al. 2019). The role of *P. spumarius* in the epidemiology of the OQDS was evaluated and supported by numerous studies on the ecology and transmission efficiency of this species (Elbeaino et al. 2014; Saponari et al. 2014; Cornara et al. 2016, 2018). Moreover, the ability to transmit XFP has been proved, under experimental condition, also for *Neophilaenus campestris* (Fallén 1805) and *Philaenus italosignus* Drosopoulos and Remane 2000 (Cavalieri

et al. 2019). The presence of these three spittlebug species was reported also for Tuscany (Mazzoni 2005; Panzavolta et al. 2019; Gargani et al. 2021), although *P. italosignus* appears to be restricted only to the coastal area of the Province of Grosseto (Southern Tuscany) and may be absent in the Monte Argentario promontory (Gargani et al. 2021). In the latter area, *P. spumarius* and *N. campestris* are the two most abundant spittlebugs and some specimens of both species were found positive to XFM ST 87 (Gargani et al. 2021). Even though *P. spumarius* and *N. campestris* were also found positive to the other *Xylella* ST reported in Corsica, France, and Spain (Cruaud et al. 2018; Generalitat Valenciana 2020), their involvement in the transmission of the bacterium in natural areas populated by Mediterranean shrubs has never been assessed.

This study is aimed at evaluating the ability of *P. spumarius* and *N. campestris* to acquire and transmit XFM from infected to healthy plants. Their efficiency as vectors has been verified in semi-field trials, using the Italian buckthorn as experimental plant species. Then, the role of these spittlebugs in the transmission of *X. fastidiosa* in natural areas is discussed.

Materials and methods

Acquisition and transmission experiments were carried out in the demarcated area of Monte Argentario (Ministerial Decree 13/02/2018 and subsequent amendments). The entire procedure was repeated two times: from June 2020 to February 2021 and from June 2021 to February 2022. *Rhamnus alaternus* was chosen as test plant because it was one of the species of the Mediterranean maquis most frequently found infected in the Monte Argentario area (Fitosirt database https://fitosirt.regione.toscana.it) and for its availability in nurseries as small plants fitting the size of our experimental cages.

Collection of insects

Adults of *P. spumarius* and *N. campestris* were collected in June in two *Xylella*-free areas in the province of Florence (Tuscany) using sweeping nets. Specimens of both species were collected from herbaceous plants and *Cupressus sempervirens* L. trees in vineyards and their surroundings. Overall, more than 230 *P. spumarius* and 230 *N. campestris* specimens were collected in both experimental periods. Each specimen was individually placed in 1.5 mL micro vials and brought to the laboratory for the taxonomical identification. Spittlebugs were identified under a stereomicroscope, according to the most common taxonomic keys (Biedermann and Niedringhaus 2009; Drosopoulos and Remane 2000; Holzinger et al. 2003; Kunz et al. 2011; Wilson et al. 2015). In addition, morphological identifications were confirmed by DNA sequencing performed on randomly selected specimens using the 5' region of mitochondrial cytochrome oxidase I gene as follows: DNA was extracted from dissected head using QIAmp DNA extraction Kit (QIAGEN) following the manufacturer instructions; the final elution step was performed in 50 µL of AE buffer supplied with the kit. Amplification was obtained using LCO1490 and HCO2198 (Folmer et al. 1994) primers for *Neophilaenus* sp. and LCO-Philaenus (5'-TCTACTAATCACAAAGATATCGG-3'; this work) and HCO2198 (Folmer et al. 1994) primers for *Philaenus* sp. PCR reaction was performed in 50.0 µL total volume containing 25.0 µL of DreamTaq Hot Start PCR Master Mix (2X) (ThermoFisher Scientific), 0.6 µM of each primer and 50 ng of DNA. The resulting amplicons were purified and sequenced using SeqStudio genetic analyser (Applied Biosystems) following the suggested protocol.

After the species identification, insects were transferred to Monte Argentario keeping them in two separated Bugdorm© cages containing potted non-infected plants supplied as food source.

Acquisition and transmission tests

Before setting acquisition-transmission tests, 20 *P. spumarius* and 20 *N. campestris* were randomly chosen among those collected in field and analysed by qPCR (EPPO 2019; Harper et al. 2010, Erratum 2013) to confirm the absence of the bacterium.

For both *P. spumarius* and *N. campestris*, acquisition trials were performed by confining the spittlebugs on branches of naturally infected *R. alaternus* wild shrubs, located in the municipalities of Porto Ercole (42.37701N, 11.18620E) and Porto Santo Stefano (42.431764N, 11.141622E) (Fig. S1). The infection status of these plants had been assessed by the Regional Health Plant Service (RHPS—Tuscany) during the annual surveillance program, and the presence of *X. fastidiosa* in the selected branches was confirmed by molecular analysis according to PM7/24 (4) (EPPO 2019; Harper et al. 2010, *Erratum* 2013).

The procedure to determine the Acquisition Access Period (AAP) and the Inoculation Access Period (IAP) was adopted from Cavalieri et al. (2019) with a few modifications to optimize the acquisition and transmission efficiencies: (a) 120 h instead of 96 h AAP; (b) a higher number of specimens per test plant in IAPs.

For each branch, 35 spittlebugs were caged in a fine mesh net sleeve for an AAP of 120 h. Overall, a total of 175 specimens for both *P. spumarius* and *N. campestris* (35 spittlebugs × 5 branches) were used for the AAP in both years. The infected plants were destroyed at the end of the AAP, as requested by current legislation.

Ten *R. alaternus* potted plants were tested for the absence of *X. fastidiosa* using the qPCR protocol reported in PM7/24 (4) (EPPO 2019) and used as receiving host for the transmission tests. Each plant was individually placed in a Bugdorm© cage with the insects previously exposed to the AAP, as shown in the Tables 1 and 2. Different numbers of caged specimens were due to the natural mortality occurred during the AAP.

Five cages for each spittlebug species were set up and maintained at room temperature and natural lighting. *Rhamnus alaternus* plants were watered once a week at field capacity. Insects were allowed to feed freely on tested plants for 96 h as IAP. In total 159 *P. spumarius* (ranging from 29 to 35 specimens per cage) and 140 *N. campestris* (from 18 to 35 specimens per cage) were used for the inoculation test in 2020, while 124 *P. spumarius* (from 21 to 32 specimens per cage) and 118 *N. campestris* (from 21 to 27 specimens per cage) were used in 2021. In all the IAP experiments, a higher number of insects per test plant was used respect to the procedure by Cavalieri et al. (2019), to increase the probability of transmitting the bacterium.

At the end of the IAP, both dead and alive insects were removed from the cages and stored individually in 96% ethanol. DNA was extracted from insect heads following the same protocol previously described (EPPO 2019; Harper et al. 2010, Erratum 2013). Every two months leaves and branches were pruned from the four sides of each *R. alaternus* plant until the end of the experiment, when plants were destroyed and roots, stem, twigs, and leaves were separately collected. All these plant portions were analysed for assessing the presence of the bacterium. In the 2021 experiment, the final sampling of plant organs was brought forward to December 2021 instead of February 2022 for plants tested with *P. spumarius*, since all the plants were dead. For this reason, some samples were taken from dead plants. DNA from plant material was extracted using DNeasy Plant Kit (QIAGEN) following the protocol suggested by the manufacturer. The final elution step was performed in 200 µL of AE buffer supplied with the kit.

Insects and plants were analysed performing qPCR and assuming 32 and 35 cycles as cut-off threshold limits for plants and insects respectively (EPPO 2019; Gargani et al. 2021). Plants were considered infected by the bacterium when at least one of the examined portions gave positive results. The quantification of bacterial load in insects and plants was not assessed through qPCR due to the lack of a bacterial culture of XFM. Limitations in the temporary laboratory's equipment and its set up hampered the isolation of the bacterium. Moreover, XF could not be provided by authorized laboratory due to concomitant COVID-19 restrictions.

All the experimental procedures are shown as a workflow in the Fig. 1.

Table 1 Summary of the results obtained from June 2020 to February 2021: number of insects positive to *Xylella fastidiosa* subsp. *multiplex* ST87 and respective C_t values assessed after the inoculation access period in each experimental cage; C_t values of the same bacterium ST obtained from different portions of *Rhamnus alaternus* and infection status of the whole plant

2020–2021 trial		Insects			*Rhamnus alaternus* plants							
Spittlebug species	Cage label	N	Positive specimens		Assessed C_t in plant organs							Resulting plant infection status
			N	C_t	Aug	Oct	Jan	Feb				
					Branches and leaves			Roots	Stem	Twigs	Leaves	
Philaenus spumarius	A	34	0	–	–	–	–	–	–	–	–	No
	C	35	3	33.79	–	–	–	–	–	–	–	No
				33.87	–	–	–	–	–	–	–	
				28.56	–	–	–	–	–	–	–	
	E	30	0	–	–	–	–	–	–	–	–	No
	G	29	0	–	–	–	–	–	–	–	–	No
	I	31	1	28.21	–	–	–	–	–	–	–	No
Neophilaenus campestris	B	35	1	24.03	28.50	26.90	27.37	34.04	33.79	–	29.31	Yes
	D	23	0	–	–	–	–	–	–	–	–	No
	F	34	1	27.33	26.04	25.80	23.42	–	32.10	–	27.90	Yes
	H	18	0	–	–	–	–	–	–	–	–	No
	L	30	0	–	–	–	–	–	–	–	–	No

A C_t value of 35 and 32 was assumed as cut-off limit respectively for insects and plants positivity to the bacterium. Plants were considered infected when at least one of the analysed portions gave positive results

Table 2 Summary of the results obtained from June 2021 to February 2022: number of insects positive to *Xylella fastidiosa* subsp. *multiplex* ST87 and respective C_t values detected after the Inoculation Access Period in each experimental cage; C_t values of the same bacterium ST obtained from different portions of *Rhamnus alaternus* and infection status of the whole plant

2021–2022 trial		Insects			*Rhamnus alaternus* plants							
Spittlebug species	Cage label	N	Positive specimens		Assessed C_t in plant organs							Resulting plant infection status
			N	C_t	Aug	Oct	Dec	Dec 2021[a] or Feb 2022				
					Branches and leaves			Roots	Stem	Twigs	Leaves	
Philaenus spumarius	A	32	1	28.76	27.04	–		–[b]	–[b]	30.74[b]	–[b]	Yes
	B	21	4	26.11	28.62	25.71		–[b]	–[b]	29.87	–[b]	Yes
				28.00								
				29.72								
				26.95								
	C	23	1	26.04	–	–		–[b]	–[b]	31.50[b]	–[b]	Yes
	D	24	1	28.63	27.61	28.74		–[b]	–[b]	–[b]	–[b]	Yes
	E	24	1	26.99	26.16	–		–[b]	–[b]	–[b]	–[b]	Yes
Neophilaenus campestris	F	21	0	–	–	–	–	–	–	–	–	No
	G	27	0	–	–	–	–[b]	–[b]	–[b]	–[b]	–[b]	No
	H	22	0	–	–	–	–	–	–	–	–	No
	I	27	1	25.81	–	29.79	–	31.77	27.07	–	30.82	Yes
	L	21	0	–	–	–	–	–	–	–	–	No

A C_t value of 35 and 32 was assumed as cut-off limit respectively for insects and plants positivity to the bacterium. Plants were considered infected when at least one of the analysed portions gave positive results

[a]The final sampling of plant organs was brought forward to December 2021 for plants tested with *Philaenus spumarius* since all the plants were dead

[b]The sample was taken from a dead plant

Data analysis

Data collected during the two experimental years were cumulated for statistical analysis. The Chi-square test (Yates' correction for continuity) was performed to compare the survival and the acquisition rate observed for the two spittlebug species at the end of the IAP. Statistical significance was accepted for p-values < 0.05 level.

Since, a multiple-vector transfer experimental design was applied in this study, the Swallow's formula $\widehat{p} = 1 - (1 - H)^{\frac{1}{k}}$ was used to estimate the probability of transmission of *X. fastidiosa* by a single vector ($\widehat{p}$). This probability depends on the proportion of infected plants (H) and on the number of tested vectors per plant (k) (Swallow 1985). Finally, the Chi-square test was used also to compare the probability of transmission estimated for *P. spumarius* and *N. campestris*. Statistical analyses were performed using PAST 4.0 (Hammer et al. 2001).

Results

At the end of the IAP, most of *P. spumarius* specimens were alive, with a survival rate of 90.81%; while only the 56.59% of *N. campestris* specimens survived, showing a significant difference in the viability of the two spittlebug species ($\chi^2 = 81.398$; df = 1; $p < 0.05$).

Both insect species were able to acquire and transmit the bacterium XFM ST87 from infected to healthy *R. alaternus* plants, as shown in Table 1 and Table 2, with acquisition rates of 4.24% for *P. spumarius* and 1.16% for *N. campestris* (Table 3). There was no significant difference in mean acquisition rates for these two spittlebug species ($\chi^2 = 3.669$; df = 1; $p = 0.05$).

Five out of ten *R. alaternus* plants were found infected after the exposition to AAP-*P. spumarius*, while AAP-*N. campestris* infected three of the ten tested plants. The estimated probability (Table 3) of transmission by a single *P. spumarius* ($\widehat{p} = 0.024 \pm 0.001$) was higher than that expected for *N. campestris* ($\widehat{p} = 0.014 \pm 0.001$); nevertheless, no statistically significant differences in the mean transmission probabilities were observed between the two spittlebug species ($\chi^2 = 35.007$; df = 1; $p > 0.05$).

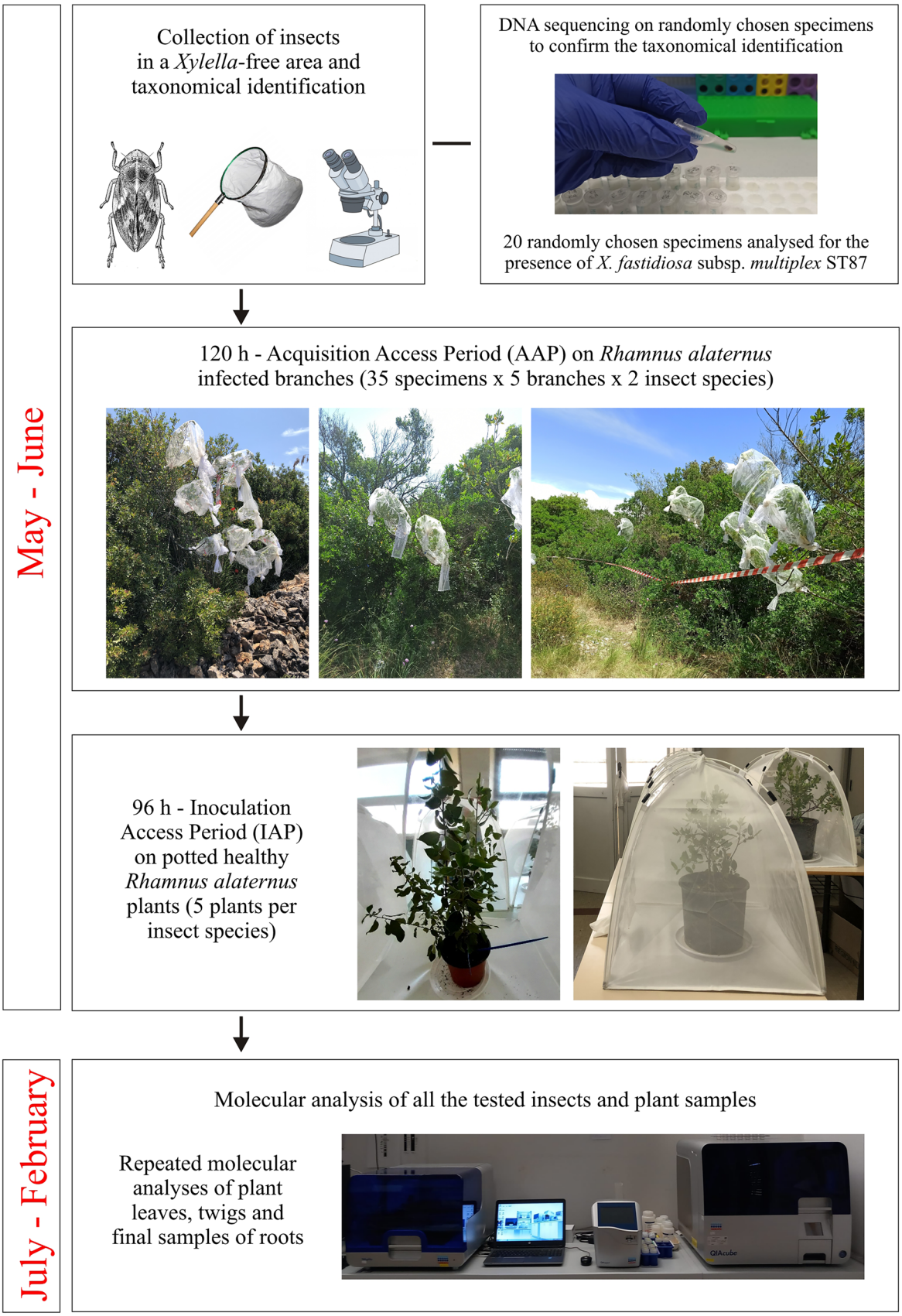

Fig. 1 Materials and methods workflow. Experimental procedures were repeated twice, from June 2020 to February 2021 and from June 2021 to February 2022

Table 3 Acquisition and transmission rates observed for *Philaenus spumarius* and *Neophilaenus campestris*

Spittlebug species	Acquisition			Transmission			$\hat{p}$*
	Total specimens	Positive specimens	Rate %	Plants exposed	Positive plants	Rate %	
Philaenus spumarius	283	12	4.24	7	5	71.4	0.024 ± 0.001
Neophilaenus campestris	258	3	1.16	3	3	100	0.014 ± 0.001

Only plants exposed to infected vectors were considered for transmission. $\hat{p}$ = Estimated probability of transmission by a single insect according to Swallow (1985) ± variance. Each test plant was considered as exposed to a mean number of specimens (respectively: PS = 28.3; NC = 25.8)

Discussion

The sequence type 87 of XFM was reported in Monte Argentario area primarily on Mediterranean shrubs and trees (Marchi et al. 2018; Saponari et al. 2019). Among these plants, the Italian buckthorn represents one of the species most frequently found infected after the outbreak was discovered (Fitosirt database https://fitosirt.regione.toscana.it).

Recent faunistic studies on the Auchenorrhyncha of Monte Argentario stated that *P. spumarius* and *N. campestris* were the two most abundant potential vectors occurring in this area (Gargani et al. 2021). Moreover, these are the only two species that have been found positive to the *X. fastidiosa* strain causing scorch diseases in Tuscany (Gargani et al. 2021; Fitosirt database https://fitosirt.regione.toscana.it). In the present study, the competence of *P. spumarius* and *N. campestris* in the acquisition and transmission of the XFM ST87 from and to *R. alaternus* plants was evaluated in semi-field experiments. While, the ability of both spittlebugs to transmit XFM ST87 to healthy plants was evidenced, results highlighted a low transmission efficiency for both species.

P. spumarius showed a significantly higher rate of survival on *R. alaternus* plants than *N. campestris.* The meadow spittlebug is well-known to be highly polyphagous, with hundreds of plant species reported as hosts (Weaver and King 1954; Yurtsever 2000; Cornara et al. 2018). On the other hand, *N. campestris* displays a narrower host range than that of *P. spumarius:* juveniles are primarily associated to monocots, while adults show a marked preference for conifers (Whittaker 1971; Nickel 2003; Mazzoni 2005; Lago et al. 2021). The high mortality of *N. campestris* may be explained by a possible unsuitability of *R. alaternus* as food plant for this spittlebug species. However, the survival rate observed in *N. campestris* is quite comparable to those recorded on *Olea europaea* L., *P. mirtyfolia* L., and *Catharanthus roseus* G. Don (Cavalieri et al. 2019). Low acquisition rates (< 5%) were observed for both *P. spumarius* and *N. campestris* in our experiment, even though the ability of the two spittlebugs to acquire the bacterium from *R. alaternus* was demonstrated. For these insect species the acquisition of XFM seems to be less efficient than that recorded for XFP infecting other source plants, even if the duration of the AAP in this study was longer in comparison to the research by Cavalieri et al. (2019). As a matter of fact, *P. spumarius* showed an acquisition rate > 15% when field-grown olive plants were used as source of XFP (Cavalieri et al. 2019). Likewise, *N. campestris*'s performance in the same experimentation was much better (acquisition rate > 5%) than the rate recorded in our study for XFM infecting wild *R. alaternus* trees. Again, higher acquisition rates by *P. spumarius* were observed after 48 h AAP on *C. roseus* (19.6%) and *P. mirtyfolia* (21.6%) infected by XFP (Bodino et al. 2022).

It has already been documented that the transmission efficiency of a vector species can vary depending on the bacterium strain and source plants. For instance, *P. spumarius* showed a higher efficiency in transmitting *X. fastidiosa* to grapevine and almond when it acquired the bacterium from grapevine instead than from almond (Purcell 1980). Another *X. fastidiosa* vector, the glassy-winged sharpshooter *Homalodisca vitripennis* (Germar 1821), appeared to be more efficient in the transmission of *X. fastidiosa* subsp. *fastidiosa* to grapevine than to almond, thus playing a major role in the epidemiology of the Pierce's disease of grapevine in comparison to the spread of Almond Leaf Scorch (Almeida and Purcell 2003). Moreover, this sharpshooter successfully transmitted XFM from almond to almond, but not to grapevine (Lopes et al. 2009). Finally, considerable differences in the transmission efficiency of *X. fastidiosa* subsp. *fastidiosa* by *H. vitripennis* were observed also according to the sequence type of the bacterium (Lopes et al. 2009).

At the end of our experiment, a few spittlebugs (of both species) that have acquired *X. fastidiosa* were able to infect healthy *R. alaternus* plants. Interestingly, when a single *N. campestris* was positive to the bacterium the infection of the recipient plant always occurred. This pattern was not observed for *P. spumarius*. We cannot exclude that this situation is due to the low number of compared specimens, so further tests could increase statistical robustness. However, the estimated probability to transmit the bacterium appeared to be low and did not significantly differ between *P. spumarius* (2.4%) and *N. campestris* (1.4%). Overall, the transmission probability for *P. spumarius* appears lower than that observed for the transmission of XFP to

olive trees, where the probability reached 7.2% (Cavalieri et al. 2019). On the other hand, it seems that there is a higher possibility of *N. campestris* infecting *R. alaternus* than infecting olive trees (Cavalieri et al. 2019). In any case, estimated probabilities for both *P. spumarius* and *N. campestris* seem to be lower than those calculated for other proved vectors of XFM, which are the sharpshooter vectors of the Plum Leaf Scald (Müller et al. 2021).

In conclusion, *P. spumarius* and *N. campestris* can transmit the XFM ST87, albeit the efficiency of *P. spumarius* seems to be lower than that recorded in the transmission of XFP ST53. In the case of *N. campestris,* this difference does not appear particularly remarkable.

In addition to the previously discussed factors, the number of bacterial cells in infected xylem vessels could affect the efficiency of the vector (Almeida et al. 2005; Lopes et al. 2009; Daugherty et al. 2010). Although the infection status of tested insects and plant material was assessed during our experiments, the content of bacterial cells in infected samples was not quantified. So, we cannot exclude that the low acquisition rate and the low transmission probability observed in our material resulted from a small quantity of bacterial cells in source plants and tested spittlebugs. As a matter of fact, acquisition and inoculation trials from and to grapevine demonstrated that a higher amount of *X. fastidiosa* in the source plant induced a greater percentage of transmission success by sharpshooter leafhoppers (Hill and Purcell 1997).

This work constitutes the first assessment of *P. spumarius* and *N. campestris* ability to transmit XFM ST87, detected in Tuscany, to *R. alaternus*, a common bush in the Mediterranean scrub. Although these findings are not conclusive, they are relevant for the ongoing *X. fastidiosa* outbreaks in Italy and Europe as well as in United States and worldwide. As a matter of fact, our results provide new accounts on the role of two polyphagous insect vectors in spreading a quarantine organism which is lethal to a huge number of wild and cultivated plant species. Notwithstanding these preliminary outcomes, further studies are necessary to evaluate the roles played by the source and the recipient plant species, as well as the relationship between pathogen isolate—vector species—host plant in order to better understand the *X. fastidiosa* pathosystem involving the subsp. *multiplex.*

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Author contributions AN, EG, and IC—conceived and designed research. AN, EG, AS, II, DR, PS, and IC—conducted experiments. EG, PFR, and PS—contributed equipment and/or analytical tools. AN, AS, II, and IC—analysed data. AN, EG, AS, IC, and II—wrote the first draft. AN, EG, PS, and IC—reviewed the manuscript. All of the authors read and approved the manuscript.

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Declarations

Conflict of interests The authors have no relevant financial or non-financial interests to disclose.

Ethical approval No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with unregulated insect species, the two spittlebugs *Philaenus spumarius* and *Neophilaenus campestris*.

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