



Investigating dispersal abilities of Aphrophoridae in European temperate regions to assess the threat of potential *Xylella fastidiosa*-based pathosystems

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Abstract

Modeling the potential spread of *Xylella fastidiosa* can document contingency plans in northern Europe, so far uncolonized by the bacterium. Through mark–release–recapture (MRR) and flight-mill experiments, the flight capacity of two potential vectors for temperate Europe was studied: *Philaenus spumarius*, the reported southern European vector, and *Aphrophora salicina*, a xylem-specialist feeding on potential host plants of *X. fastidiosa*. *Aphrophora salicina* displayed significantly better flight performances than *P. spumarius*. In flight-mills, the average distance flown was, respectively, 623 m vs. 102 m and the maximal distance flown was 6.16 km vs. 1.54 km in 2.5 h. In MRRs, *A. salicina* travelled more than 30 m in a single flight, with a maximal interception distance of 80 m after two days, highlighting that dispersal is driven by connectivity and host plant quality. *Philaenus spumarius* mainly jumped, with 1 m movements in length and a maximal interception distance of 32 m in 27 days. Models estimated *P. spumarius*' daily mean dispersal at 1.5 m and *A. salicina*'s at 3.5 m. Although only a small part of the population moves over very long distances, this pool of efficient insects could already be sufficient to effectively spread an epidemic. As Salicaceae have been reported as host plants of the bacterium, the association of Aphrophoridae and Salicaceae in riparian areas could create bacterium reservoirs in corridors allowing for transportation over medium to long distances.

Keywords *Aphrophora salicina* · *Philaenus spumarius* · Mark–release–recapture · Flight-mill · Insect vector · Salicaceae

Key message

- Local data are lacking to model the potential spread of *Xylella fastidiosa* in northern Europe.
- The flight capacity of two potential vectors, *Philaenus spumarius* and *Aphrophora salicina*, was analyzed.
- The specialist *A. salicina*, driven by the distribution of Salicaceae, flies farther than the generalist *P. spumarius*.
- A 1.54 km flight-mill flight in 2.5 h was run by *P. spumarius*, versus 6.16 km by *A. salicina*, and the daily mean dispersal was estimated at 1.5 m for *P. spumarius* and 3.5 m for *A. salicina*.
- Salicaceae trees are forming linear corridors that could allow the bacterium to move over medium and long distances.

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Introduction

The gammaproteobacterium *Xylella fastidiosa* (Xanthomonadaceae; Wells et al., 1987), one of the most harmful phytopathogens worldwide, constitutes a threat to the economy and the health of the European flora (EFSA, 2019a). Endemic to Americas, it causes major diseases on crops such as grapevine, citrus and almond (EFSA, 2018). A strain was first identified in Europe in olive groves of Southern Italy (EFSA, 2013; Saponari et al. 2013). Since then, surveys carried out in Europe have led to the identification of several other strains in different areas such as Tuscany (Italy), Corsica, mainland France, mainland Spain, the Balearic Islands and Portugal (Denancé et al. 2017; Olmo et al. 2017; EFSA, 2019a; Saponari et al. 2019). The diversity of the discovered strains suggests multiple independent introductions (Landa et al. 2020; Dupas et al. 2022). A peculiarity of *X. fastidiosa* is that each affected area is characterized by its own pathosystem, with its own range of insect vectors, host plants and environmental conditions (Sicard et al. 2018).

Any xylem-feeding insect species (Hemiptera, Auchenorrhyncha) is a potential vector (Almeida et al. 2005). The bacterium colonizes the anterior parts of the insect's digestive system (i.e., the foregut) in a persistent way (Almeida et al. 2005). The insect vectors identified so far belong mainly to two different families: the Cicadellidae (sharpshooter leafhoppers) and the Aphrophoridae (aphrophorid spittlebugs) (Redak et al. 2004; EFSA 2019c). In America, insect vectors are mainly sharpshooters (Severin 1950; Almeida et al. 2005), while in Europe aphrophorid spittlebugs constitute a large pool of potential vectors (EFSA 2015). Three spittlebug species have been identified as vectors in Europe: *Philaenus spumarius* Linnaeus, 1758, *Philaenus italosignus* Drosopoulos and Remane, 2000 and *Neophilaenus campestris* (Fallén, 1805). The sharpshooter *Euscelis lineolatus* Brullé, 1832 (Cicadellidae) was also found positive to the bacterium in Italy, but its role as vector has not been demonstrated (Elbeaino et al. 2014; Ben Moussa et al. 2016).

To colonize a new territory, the bacterium must first be introduced via infected vectors or contaminated plant material (EFSA, 2018); then, it has to be transmitted to a host plant by insect vectors, which also promote short-range dispersal (Almeida 2016). Once *X. fastidiosa* has established in a new area, its eradication is complex (Strona et al. 2017), if not impossible, partly because there is no effective curative treatment to date (EFSA, 2019b). Prevention and early detection programs are the most efficient strategies to avoid outbreaks. Modeling is a powerful tool to articulate the interactions within the *X. fastidiosa* complex system, allowing to run scenarios

and to implement preventive measures. However, data are missing to run models of bacterial potential spread in northern Europe, as the vast majority of studies and available models have been elaborated based on data from the Mediterranean region (EFSA, 2018). Knowledge gaps must be addressed for other European regions where the bacterium has not yet been in contact with local fauna and flora. Characterization of the potential insect vector population in such areas is therefore essential, including the identification of the main potential vector species, and the study of their abundance, feeding preferences and mobility (Occhibove et al. 2020).

Although all xylem-feeding insects might transmit the bacterium, only some must be considered as real threats (Almeida et al. 2005). EFSA reported the relative importance of several insect species that could be efficient vectors in Europe based on three criteria: polyphagy, abundance and geographical distribution throughout the continent (EFSA 2015), and pointed out five insects (*Aphrophora salicina* (Goeze 1778), *Aphrophora alni* Fallén 1805, *Cicadella viridis* (Linnaeus 1758), *Cercopis vulnerata* (Rossi 1807) and *P. spumarius*) for which distribution, adult phenology and host plants were studied (Hasbroucq et al. 2020). Their relative importance as potential vectors should therefore be determined by means of appropriate studies, including those aimed at unveiling their dispersal capacities. As *Salix alba*, *Salix caprea* and *Populus tremula* (Salicaceae) were identified as potential hosts for the bacterium (Casarin et al., submitted; available on BioRxiv), *A. salicina* was selected for further investigation because of its connection with the *Salix* genus (Nickel and Remane 2002; Hasbroucq et al. 2020). This insect species is very abundant on *Salix* spp., especially in riparian environments (Hasbroucq et al. 2020), areas which were pointed out as important reservoirs of *X. fastidiosa* in California (Purcell 1974; Costello et al. 2017). On the other hand, *P. spumarius* was selected for its important and well-documented status of vector in Europe (Cornara et al. 2016; Cruaud et al. 2018; López-Mercadal et al. 2021), for its polyphagy, its wide distribution in all the Palearctic region (comprising northern Europe) and for its ability in colonizing most ecological regions and types of environment (Hasbroucq et al. 2020).

Spittlebugs adults are often considered poor flyers that prefer jumping instead of flying (Weaver and King 1954; Cornara et al. 2018). The first studies quantified the dispersion of *P. spumarius* at around one hundred meters per year (Weaver and King 1954; Halkka et al. 1967). Bodino et al. (2021) recently studied the dispersal of *P. spumarius* with mark–release–recapture (MRR) experiments and established a dispersal kernel where 98% of the insects remained within a 400 m radius during a two months period, but with variation according to the diverse agrosystems studied. Lago et al. (2021a) also studied *P. spumarius*' flight performance,

with a flight-mill device recording a maximal single flight of 5.5 km in 5.4 h, largely out compassing the previous observations (Weaver and King 1954; Halkka et al. 1967; Bodino et al. 2021). On the other hand, there is no information on the flight capacity of *A. salicina*, and only a few studies on Aphrophoridae other than *P. spumarius* have been undertaken. In a MRR assay on *N. campestris* (Aphrophoridae), Lago et al. (2021b) collected a specimen after 35 days more than 2 km from the release point, which suggests that flight abilities could be quite different between species within the same family. Inferring the dispersion of potential insect vectors in understudied areas based on current knowledge is therefore not sufficient.

To improve knowledge on *P. spumarius* and *A. salicina* in European northern temperate areas, the flight abilities of *A. salicina* and *P. spumarius* were studied through MRR and flight-mill experiments. Males and females, at different physiological stages (early adult life, sexually immature, mated, egg-laying, end of life), were tested and compared on flight-mills, which allows easy testing of the impact of abiotic and biotic factors (e.g., sex, age, humidity, temperature) on flight performances in controlled conditions (Lago et al. 2021a). The physiological stages were checked over the season through dissection of spermathecae and observation of ovary maturity, together with field surveys. In parallel, MRR experiments in the wild (Franklin and Grégoire 1999; Franklin et al. 2000; Meurisse et al. 2008) were performed in different urban and semi-natural environments and at different period of the year to measure mobility under different environmental conditions and seasonal changes. Data coming from both experiments were finally pooled together in a model to determine and compare the flight abilities of both species.

Materials and methods

Mark–release–recapture experiments

Adult insects were caught, marked with fluorescent powder, released at a given point directly after marking and finally monitored on the following days. The experiments were carried out from June to September, for which a summary table is provided in the Supplementary file 1 (Table S1). The survey took place over three summer seasons from 2019 to 2021: in 2019, only *A. salicina* was studied in June and in August; in 2020, both species were studied in July, August and September; in 2021 only *P. spumarius* was studied in late June. 2.1.1 *The sites.*

The experiments were carried out i) in an urban environment, where vegetation is simplified and crossed by roads where car, pedestrian and bicycle traffic are present, and ii) in a semi-natural environment (natural reserve) where

vegetation is managed for wildlife preservation. The sites were chosen for accessibility, insect abundance and vegetation type (a mix of ground vegetation, Salicaceae trees and shrubs). A map of each site was drawn (Fig. 1) with a description of the trees (Salicaceae vs. non-Salicaceae). For *A. salicina*, the four different sites (one urban and four semi-natural) were: A. urban, along the Woluwe boulevard in Brussels; C. semi-natural, in the natural reserve of the decanters of the sugar factory of Genappe; D. semi-natural, on the site of the marshes of Neuville in Hensies; E. semi-natural, within a natural site of La Louvière, Garocentre. For *P. spumarius*, two sites were selected (one urban and one semi-natural): B. urban, along the Woluwe boulevard in Brussels, a few hundred meters from the study site of *A. salicina*; C. semi-natural, in the natural reserve of the decanters of the sugar factory of Genappe.

Capture, mark and release

A total of 200 to 1000 insects were collected with nets and mouth aspirators in each site (Table S1). Once captured, they were placed in 250 ml perforated vials in batches of 50 specimens for *A. salicina* and of 100 for *P. spumarius*. The insects were then marked with fluorescent powder (Radglo®QF- 17Radiant Color, 5 µm) by adding 1 g of powder per vial and by gently shaking. A different color was used (Table S1) to be able to differentiate on a same site the insects released at different times. They were released at a single spot from a ziggurat-like take-off platform placed on a tray to collect the non-flyers. The platform was placed on the ground for the release of *P. spumarius*, mostly on herbaceous layer, and on a pole of 1 m 20 high for *A. salicina*. The release was done one vial at a time, from 10:00 am to 4:00 pm. In total, 6340 specimens of *A. salicina* and 3526 *P. spumarius* were released. The first flight or jump from the platform was observed during the first hour after the release, for which the orientation and the distance were reported. To allow a quick assessment of the distance traveled, stakes were planted as markers at different locations from the release point (0.5, 1, 2, 5, 10 and 30 m) and the interval in which the insect landed was recorded.

Observations and recaptures through time

The monitoring of the travelled distance was conducted in two phases: a first phase of daily observations during seven days, followed by a recapture phase of one day one month after each release (Table S1). A floristic inventory was conducted within a 100 m area around the release point: the space was segmented into numbered quadrats and the tree species were identified. During the seven days post-release (observation phase), monitoring of the insects without recapture was carried out by visually screening the entire 100 m

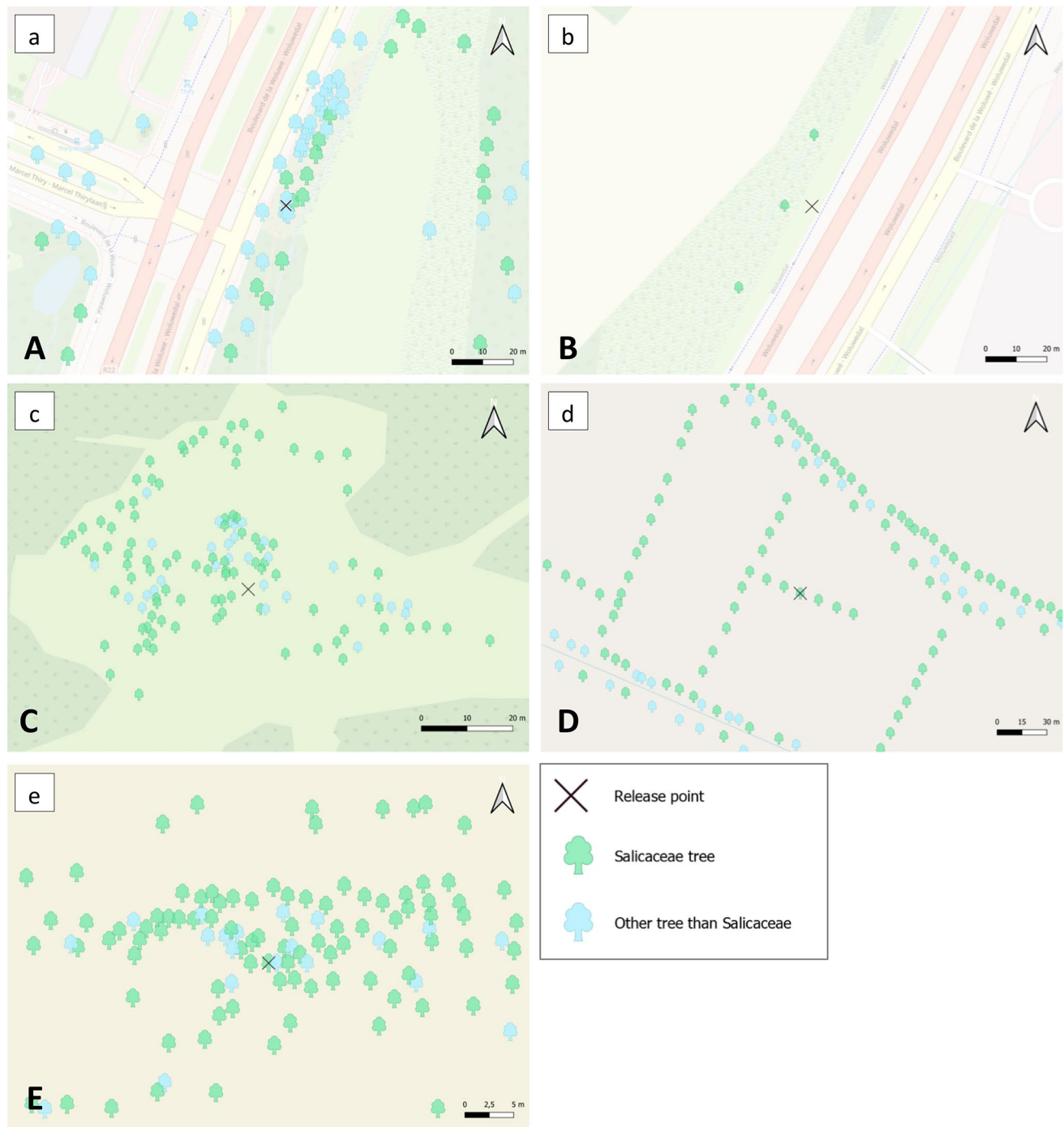


Fig. 1 Maps of the study sites for the mark–release–recapture experiments performed with *Aphrophora salicina* and *Philaenus spumarius* in urban and semi-natural environments. **a.** Map of site A (urban environment; Brussels; 50.8537, 4.4445). **b.** Map of site B (urban environment; Brussels; 50.8588, 4.4486). **c.** Map of site C (semi-natural environment; Genappe; 50.6040, 4.4364). **d.** Map of site D

(semi-natural environment; Hensies; 50.4468, 3.6745). **e.** Map of site E (semi-natural environment; La Louvière; 50.4981, 4.1886). *Aphrophora salicina* was released in the sites A, C, D and E and *P. spumarius* in the sites B and C. Maps were produced by S. Hasbrouck with the software QGIS © 3.18.1 “Zürich.”

area. For each marked insect sampled, its distance from the release point was measured. During the second phase, the 100-m site was searched systematically with a sweeping net.

For tree captures, a net mounted on a 6-m-long telescopic pole was used. The insects were analyzed under a 366 nm UV lamp (Merck® KGaA) to check for traces of fluorescent

powder. During these two monitoring phases, searches for marked insects were conducted with sweeping nets between 100 and 200 m to check for further movement. In addition, yellow sticky traps were placed between 100 and 300 m from the releasing point in the urban site (Brussels). However, they were not allowed in the natural sites to preserve the wildlife. The traps were visited daily during the observation phase and then during recaptures, and replaced monthly.

Flight-mill experiment

Flight-mill experiments were conducted as described by Maes et al. (2014). The rotating-arm length was 7.96 cm for *P. spumarius* and 15.9 cm for *A. salicina*, corresponding to distances covered in one complete rotation of 0.5 m and 1 m, respectively. In total, ten flight-mills were operated simultaneously.

The insects tested were collected in the field and were used directly after collection and up to four days later. During these days, they were maintained in captivity in small cages on potted Salicaceae plants with a controlled 15:9 photoperiod and temperatures ranging from 19 to 23 °C. For *A. salicina*, 393 individuals were tested over three years, in June 2019, from June to September 2020 and from July to September 2021. For *P. spumarius*, 398 individuals were tested over two years, from July to September 2020 and from June to September 2021. Each insect was first sexed (Supplementary file 1 Fig. S1) and was maintained on the flight-mill for 2.5 h, and the total distance travelled during this time was measured. This time was chosen as beyond it, either mortality or a significant decreasing in the flight activity was registered. Moreover, beyond four hours of flight, a very high mortality was observed. In 2019 and 2020, the insects were kept for about 10 s on cold packs (around 3 °C) to facilitate gluing. In 2021, as we had improved in dexterity, the insects were directly attached to the mill without previous cooling.

Maturity and physiological states

Physiological states were determined based on dissections of spermathecae and ovaries of female individuals, together with behavioral observation of the insects in the fields or in laboratory microcosms, depending on the species. Given that *Aphrophora salicina* is closely associated with willows, behavioral observations in the field were facilitated. On the other hand, because of the polyphagy of *P. spumarius*, mating behavior and oviposition are more difficult to spot in the field as they can occur on a variety of plants. For this reason, several *P. spumarius* specimens were kept in captivity and monitored for their changing behaviors in microcosms, consisting of cages with *S. alba* seedlings exposed to outdoor conditions. Regarding the spermathecae state and the ovary maturity, the females previously submitted to the flight-mill experiment, were dissected and observed under a Leica MZ6 stereomicroscope equipped with a Leica DFC295 camera. About ten females per species and per month (June, July, August and September) were analyzed. The males were not dissected; their sexual maturity was aligned to the state of the spermathecae observed in females.

Five different adult stages were investigated: i. early adult life; ii. sexually immature; iii. mated; iv. egg-laying; v. end of life. A diagram and description on how these stages have been determined is provided in the Supplementary file 1 (Fig. S2). The period observed for different states of the adult life of the insects is visualized in Fig. 2. Low temperature in 2021 delayed the beginning of the adult stage, which occurred several weeks after the usual period.

Gathering MRR and flight-mill data

The data from flight-mill and MRR assays were combined in a dispersal model to estimate the potential dispersion of both species in the wild. The model was based on Robinet



Fig. 2 Different physiological states in the insect life of *Aphrophora salicina* and *Philaenus spumarius*. The adult life has two different starts corresponding to different years (Upper line=year 2020, lower line=year 2021). For *A. salicina*: i. Early adult life from May 25 to June 15 in 2019–2020 and from June 15 to June 25 in 2021; ii. Immature stage from June 16 to June 21 in 2020 and from June 26 to July 4 in 2021; iii. Mating stage from June 22 to July 15 in 2019–2020 and from July 5 to July 15 in 2021; iv. Egg-laying stage starting around

July 15; v. End of life, population drop around September 5 and last insects observed in October. For *P. spumarius*: i. Early adult life from May 10 to June 10 in 2020 and from May 30 to June 20 in 2021; ii. Immature stage from June 11 to July 24 in 2020 and from June 21 to July 31 in 2021; iii. Mating stage, from July 25 to August 20 in 2020 and from August 1 to August 20 in 2021; iv. Egg-laying stage starting around August 20; v. End of life, population drop around September 20 and last insects observed in November.

et al. (2018, 2019), with parameters adapted with our own values for *A. salicina* and *P. spumarius*. The flight-mill assays allowed to determine the probability of an insect to fly and to estimate the daily mean dispersal distance (alpha; in meters) by fitting a dispersal kernel over the distribution of individual flight performances (with intervals of 10 m). In the model, the number of insects was fixed at 100, as well as the number of simulation days. The initial age was fixed at one day for all insects (representing the first day of their adult life); the active longevity (age_max) was determined at 153 days (one adult season) for *P. spumarius* and 121 days for *A. salicina*; the number of days needed for resting after one flight was set varying from 0 to 1 day. The distance travelled by each insect was fixed via the distance parameter, which takes a random value according to an exponential distribution with a rate equal to $1/\alpha$ considering that the insect can fly in all directions (2π) in a uniform environment (see Robinet et al. 2018, 2019).

The model was then parameterized, finding the alpha parameter that minimizes the error between the simulation results and the MRR observations of the year 2020. To do so, several parameters were adapted: the number of simulation days corresponded to the ones of the MRR experiment, and for each insect, a random age value between one and the age_max was given according to a randomized uniform distribution. Finally, the root mean square error (RMSE) was calculated between the simulation results and the MRR results, taking the average distance flown by the insects as a comparison factor. The alpha parameter that minimizes the RMSE was then selected for both *A. salicina* and *P. spumarius*. One hundred simulations of the parametrization model were run and the most frequently occurring alpha was retained and inserted in the dispersal model.

The dispersal model was run one hundred times and the distance data of the 100 insects were stored in matrices that were then used to calculate the percentage of the population that had exceeded a threshold distance for each day of the simulation. The threshold distance investigated was 50 m, the radius of the area that must be demarcated in the event of a detection of a bacterial outbreak according to the European regulation (EC, 2020). All simulations and graphics were made with the open-source software R[®] 4.0.4 (R Core Team 2021).

Statistical analysis

To study flight performance of *A. salicina* and *P. spumarius*, the probability of flight and distance travelled on flight-mill were selected as dependent variables and the effect of species, physiological states, sex and year was investigated. The probability of flight was studied through a generalized linear model (GLM) with a binomial distribution for the conditional dependent variable. Then, the general linear

hypotheses (glht) function for parametric models was run on contrast matrix ($P < 0.05$). The contrast matrix was built with the package multcomp (Hothorn et al. 2008), which tested only the combinations of interest. On the other hand, the distance travelled was processed differently according to the species. For *P. spumarius*, the log transformation of the travelled distances allowed to study the data through a classical linear regression model, running the glht function on a contrast matrix ($P < 0.05$). In the case of *A. salicina*, given that the distance data did not meet assumptions required by linear regression, a Kruskal–Wallis nonparametric test ($P < 0.05$) was used. Wilcoxon pairwise tests were then applied to compare the means of each specific group, and p-values were corrected using the Benjamini–Hochberg procedure. Then, to compare the distances flown by the two species, a nonparametric Wilcoxon test ($P < 0.05$) was used. Graphs of the travelled distances were built using the ggplot function (package ggplot2; Wickham 2016) by transforming the distance values “x” by $(\log_{10}(x + 1))$.

Regarding the first-flight data of MRR experiments, a Chi-square goodness-of-fit test ($P < 0.05$) was applied to assess the differences in orientations (N, S, E, W) for each species in each site, followed by post-hoc tests using a Bonferroni correction to adjust p-values. Concerning the distances of first flights, they were treated as interval-censored data, because precise point estimates could not be determined. A survival analysis capable of handling this type of data was chosen. More specifically, Cox models (Cox 1972) with gamma baseline were performed using the package icenReg (Anderson-Bergman 2017) to study species and site effect on dispersal in 2020 ($P < 0.05$). All analyses were made with the open-source software R[®] 4.0.4 (R Core Team 2021).

Results

Dispersion behavior analyzed by MRR experiments

First-flight behavior

The first flight, or jump, undertaken by the insects from the take-off platform highlighted behavioral differences between *P. spumarius* and *A. salicina*. The distances recorded for both species, according to the period and site, are shown in Table 1. *Philaenus spumarius* travelled shorter distances, e.g., an average of 0.8 m compared to 5.5 m for *A. salicina* in mid-July within the same site. The instantaneous risk of landing was 9.14 times higher for *P. spumarius* than for *A. salicina* (hazard ratio = 9.14, $p = 0$) in 2020.

Aphrophora salicina mainly landed in the release area either on the ground or on willows. Zigzag behavior before landing on a willow was often observed, i.e., flying while

Table 1 Data of the intercepted distances and first flights observed during the mark–release–recapture (MRR) trial for *A. salicina* and *P. spumarius* in different sites, in different periods.

Insect species	Type of site	Site code Fig. 1	Year	Release period	Average distance 1st flight (m)	Av. dist. 1st flight /site (m)	Max distance 1st flight (m)	Average cumul. distance (m)	Av. cumul dist/ site (m)	Max. dis- tance (m; day)
<i>A. salicina</i>	Urban	A	2019	End of June	–	–	–	6.3	6.4	23; 2
				Early Aug	–	–	–	6.4	–	16; 4
			2020	End of June	3.1	2.69	12	3.7	3.5	31; 7
	Semi-natural	E	2019	Early Aug	2.4	–	25	2.5	–	6; 12
				Early Sept	1.5	–	5	2.9	–	8; 23
				End of June	–	–	–	1.9	1.9	13.5; 20
<i>P. spumarius</i>	Urban	D	2019	Early Aug	–	–	–	35.4	35.4	80; 2
		C	2020	Mid-July	5.5	4.3	> 30	7.7	6.3	53; 7
				Mid-Aug	1.4	–	4	4.2	–	25; 5
				Early Sept	2.4	–	10.2	5.8	–	51; 7
		B	2020	Early July	1	0.9	3	1.5	1.5	17; 50
				Early Aug	0.7	–	1.5	1.5	–	7; 22
	Semi-natural	C	2020	Early Sept	0.8	–	2	1.3	–	4; 7
				Mid-July	0.8	0.86	2.5	1.3	1.5	17.2; 43
				Mid-Aug	0	–	0	1.5	–	6; 27
				Mid-Sept	0.9	–	2.5	1.7	–	32; 27
			2021	Mid-June	0.8	0.8	1	1.9	1.9	6; 723

The site code refers to Fig. 1: AB are urban sites; C, D, E are semi-natural sites. “–” stands for absence of observations. The cumulative distance corresponds to the sum of the distances recorded during all observation days (namely, seven days and one month after insect release). Within the column “Max distance,” the value “day” indicates how many days after release the insect was intercepted. Values in bold are the maximum values intercepted for each species taking into account any site and period

constantly changing direction and sometimes drawing circular trajectories. The furthest first flight for this species was recorded in mid-July in the semi-natural site C, where a dozen individuals traveled long distances beyond 30 m in a single flight. After this point, it was not possible to accurately measure the exact distance the insects covered, as they were lost from sight and could no longer be tracked. The instantaneous risk of landing was higher in the urban site A than in the semi-natural site C (hazard ratio = 0.66, $p < 1 \times 10^{-8}$), suggesting that *A. salicina* travelled greater distances in the semi-natural site during its first flight. Concerning the directional preference after the take-off, *A. salicina* took significantly more certain directions in both sites. In the urban site A (Chi-squared = 331.65, $df = 3$, $p < 2.2 \times 10^{-16}$), this species flew toward the North, thus in the direction of the willow alignment (Fig. 1a, site A), more frequently than toward other directions ($p < 1 \times 10^{-24}$ with East; $p < 1 \times 10^{-34}$ with West; $p < 1 \times 10^{-39}$ with South), while the East was preferred compared to the South ($p = 0.004$). Furthermore, it was observed that the insects were always turning back when approaching the main road (authors' personal observation). In the semi-natural site C (Chi-squared = 90.91, $df = 3$, $p < 2.2 \times 10^{-16}$), *A. salicina* also flew more toward the North than toward other directions ($p < 1 \times 10^{-6}$ with East; $p < 1 \times 10^{-17}$ with West; $p < 1 \times 10^{-5}$ with South), while the East was preferred compared to the West ($p < 0.001$) and the South was also preferred compared to the West ($p < 0.001$).

Philaenus spumarius' take-offs were mainly performed by jumps of ca. 1 m. Only a limited number of insects flew, for which a maximum distance of ca. 3 m was observed in early July within the urban site B. No significant differences in terms of distances were measured between the two sites (B and C). *Philaenus spumarius* systematically landed on the herbaceous vegetation near the release point. In mid-August 2020, in the semi-natural site C, the mean distance covered was 0 m, probably due to a heat wave which resulted in high mortality rates and limited dispersal. In both sites, *P. spumarius* showed a statistically significant preference in the direction taken after the take-off. In the urban site B (Chi-squared = 16.85, $df = 3$, $p < 0.001$), this species flew more toward the North, thus on the opposite side of the road (Fig. 1b, site B), compared to both the East ($p = 0.017$) and the South ($p = 0.001$). In the semi-natural site C (Chi-squared = 64.67, $df = 3$, $p < 1 \times 10^{-13}$), *P. spumarius* also flew more toward the North than in the other directions ($p < 1 \times 10^{-8}$ compared with East; $p < 1 \times 10^{-9}$ compared with West; $p < 0.001$ compared with South).

Intercepted distance monitoring

Table S2 and Fig. S3 (Supplementary file 1) comprise a summary of the number of marked insects found, as well as the evolution of the observation rate during the whole

trial period (thus, from 1 to 50 days after the release day). The information is provided for both species and for each site and period. The observation rate decreased from day to day regardless of insect species, environment, or period (Table S2 and Fig. S3). In average, it was 14.1% after 24 h, 6.7% after 7 days and 0.9% after more than 20 days for *A. salicina*. On the other hand, it was 31.3% after 24 h, 8.4% after 7 days and 0.6% after more than 20 days in the case of *P. spumarius*.

Maps showing the dispersion of the insects over time are displayed in Fig. 3 and in Supplementary file 2, showing the dispersal of *A. salicina* and *P. spumarius* in urban and semi-natural environments. The mean cumulative distances recorded during different days of observation and the maximal distances from the release point in each MRR assay are presented in Table 1. Regardless the site, period or species, no marked insects were found beyond the 100 m area surrounding the release point. No marked insects were captured on the 56 traps analyzed.

Aphrophora salicina performed the longest distance in the semi-natural site D, with an average of 35.4 m in 2019 (Table 1). This value was indeed higher than the average values recorded in the other semi-natural sites (namely, E (1.9 m in 2019) and C (6.3 m in 2020)) and in the urban site A (6.4 in 2019 and 3.5 in 2020). The maximal distance from the release point was 80 m two days after release, recorded on site D. The maps in Supplementary file 2 show that in all semi-natural sites (C, D, E), *A. salicina* dispersed in all directions regardless of the period. In contrast, in the urban site A, *A. salicina* moved along the willow alignment and was never encountered beyond the road that was parallel to the alignment (Fig. 3). The maximal distance at which *A. salicina* was observed within the willow alignment was 31 m after seven days. This species was found mainly on willows (*S. alba* and *S. caprea*), with a few individuals also observed on birch (*Betula pendula*), common ash (*Fraxinus excelsior*), maple (*Acer campestre*), alder (*Alnus incana*), blood dogwood (*Cornus sanguinea*) or in the herbaceous layer. After seven days, the insects were generally found only on willow (91.7%), and a few individuals were still reported on birch (2.6%), blood dogwood (1.9%), common ash (0.6%) or on the herbaceous layer (3.2%).

The monitored distances were shorter for *P. spumarius* than for *A. salicina* (Fig. 3). In the urban site B, *P. spumarius* was recorded at a maximum distance of 17 m after 50 days (Table 1). A dozen individuals were observed beyond the bicycle and pedestrian path, but none were recorded beyond the road (Fig. 3). In the semi-natural site C, the maximum distance recorded was 32 m, 27 days after release. In general, the insects were found to disperse in all directions. This species was generally found on the herbaceous layer (97.6%) mainly on Poaceae, Asteraceae, Fabaceae and Euphorbiaceae.

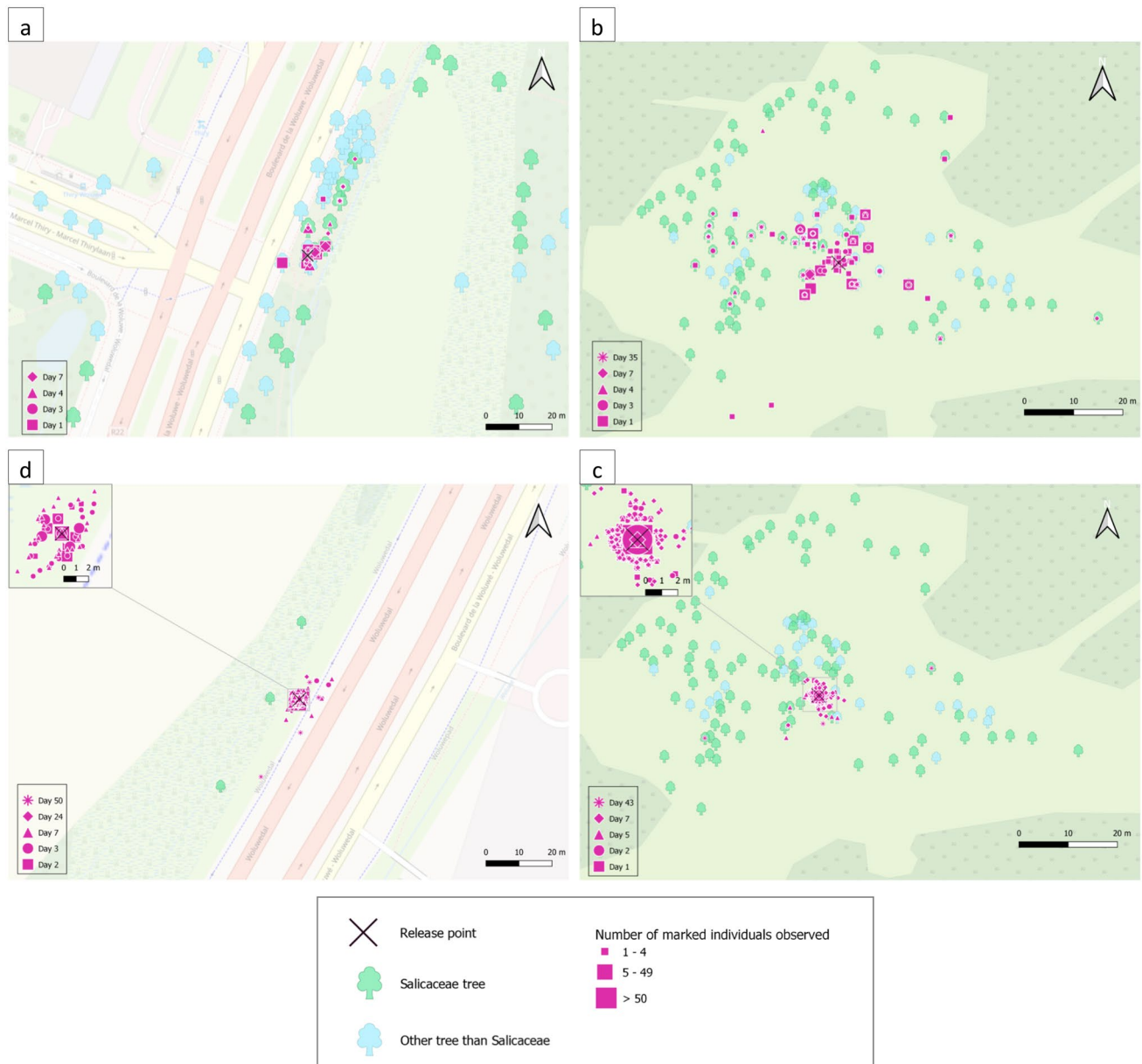


Fig. 3 Dispersion maps of *A. salicina* and *P. spumarius* after a mark–release–recapture experiment in July 2020. **a.** Dispersion of *A. salicina* in the urban site of Brussels (A in Fig. 1). **b.** Dispersion of *A. salicina* in the semi-natural site of Genappe (C in Fig. 1). **c.** Disper-

sion of *P. spumarius* in the urban site of Brussels (B in Fig. 1). **d.** Dispersion of *P. spumarius* in the semi natural site of Genappe (C in Fig. 1). Maps were produced by S. Hasbroucq with the software QGIS © 3.18.1 “Zürich.”

However, a few individuals were also observed on birch (0.2%), willows (1.1%) and bramble (*Rubus* sp.; 1.1%).

Probability of short vs. long flight distances analyzed with flight-mill experiments

Flight propensity

Flight propensity was considered only in 2021 (Table 2), as it had been significantly lower than the previous year

when the same physiological states were compared (Chi-squared = 61.84, $df = 11$, $p < 1 \times 10^{-8}$ for *A. salicina*; Chi-squared = 31.88, $df = 11$, $p < 0.001$ for *P. spumarius*) because of the sedation on ice, as reported in Fig. S4 (Supplementary file 1). 83.3% of the *A. salicina* (163 individuals out of the 198 tested) started to fly in 2021, against only 46.6% (61/131) in 2020. Similarly, 47.9% (115/240) of the *P. spumarius* flew in 2021 against 19.9% (30/151) in 2020. Nevertheless, for both years, flight propensity was significantly lower for *P. spumarius* than for *A. salicina* (for 2021:

Table 2 Summary of the data collected during flight-mill experiments for *A. salicina* and *P. spumarius* for each physiological state and sex

Species	Stage	Sex	Distance travelled in 2.5 h					Probability of flight	
			Count	Mean [m]	std [m]	Median [m]	Max [m]	Count	[%]
<i>A. salicina</i>	Early adult		243	623	827	351	6160	198	83.3
		<i>F</i>	7	48.9	87.4	11	238	–	–
		<i>M</i>	3	9.67	15	1	27	–	–
	Mating	<i>F</i>	31	967	883	692	3612	26	88.5
		<i>M</i>	31	591	586	531	2668	32	78.1
	Egg-laying	<i>F</i>	51	764	1189	302	6160	38	86.8
		<i>M</i>	77	520	638	274	3870	71	83.1
	End of life	<i>F</i>	8	748	871	446	2491	10	70
		<i>M</i>	25	682	718	488	2435	32	90
<i>P. spumarius</i>	Early adult		145	102	231	16	1542	240	47.9
		<i>F</i>	5	236	369	61	880	10	50
		<i>M</i>	5	39.5	83	1	188	19	26.3
	Immature	<i>F</i>	16	190	344	118	1427	26	61.5
		<i>M</i>	16	140	187	45.5	631	35	45.7
	Mating	<i>F</i>	28	166	330	53.5	1542	33	54.5
		<i>M</i>	13	72.8	216	3.5	788	27	37
	Egg-laying	<i>F</i>	19	68.3	162	20.5	716	26	61.5
		<i>M</i>	17	22.2	28.2	11	90	34	41.2
	End of life	<i>F</i>	14	16.8	25.2	5	80.5	22	45.5
		<i>M</i>	9	65.9	156	2	476	8	62.5

For the distance travelled, the data from different years were combined (2019, 2020 and 2021), as no significant difference between them was measured. For probability of flight, the data concerned only the year 2021. “–” stands for absence of data

Chi-squared = 62.05, $df = 1$, $p < 1 \times 10^{-14}$; for 2020: Chi-squared = 23.14, $df = 1$, $p < 1 \times 10^{-5}$). Sexes and physiological states did not affect flight propensity for both species.

Distance flown and long-flight ability

The frequency distribution of the flight distances travelled in 2.5 h by *A. salicina* and *P. spumarius*, is represented in Fig. 4. Mean-, median- and maximal distances are shown in Table 2. Data are grouped independently of the year, as no significant difference between different years was measured. A higher proportion of *A. salicina* specimens undertook longer flights than *P. spumarius*, and *A. salicina* also flew further, as supported by the shape of the cumulative frequency curve in Fig. 4 and the fact that this curve continues further for this species than for *P. spumarius*, corresponding to a greater maximum travelled distance. The travelled distances within the time period of 2.5 h were statistically different between the two species ($W = 27,325$, $p < 2.2 \times 10^{-16}$). *Aphrophora salicina* travelled, on average, 623 m in 2.5 h, while *P. spumarius* travelled an average of 102 m (Table 2). The median distances travelled by the two species were rather different, being 351 m for *A. salicina* and 16 for *P. spumarius*. The maximal distance recorded for *A. salicina* was 6.16 km,

which was covered by a female in the egg-laying phase in early September 2021. The maximal distance for *P. spumarius* (i.e., 1.54 km travelled in 2.5 h) was covered by a mating female at the end of June 2020 and was four times shorter than the distance covered by the *A. salicina* female.

Impact of sex and physiological states

Travelled distances by *A. salicina* and *P. spumarius* are represented in Fig. 5. Sex and physiological states significantly affected the travelled distances for both species (*A. salicina*: Chi-squared = 25.49, $df = 7$, $p < 0.001$; *P. spumarius*: Chi-squared = 21.43, $df = 9$, $p < 1 \times 10^{-4}$). In the case of *A. salicina*, the “emergent” groups tended to fly shorter distances in 2.5 h compared to the individuals in other physiological stages; even so, the “emergent” group of females statistically differed only when compared to the “mating” group of females ($p = 0.004$). While Wilcoxon pairwise tests did not reveal major differences between males and females in the same physiological state, the values in Table 2 are slightly and consistently greater within the group of females than within males, except when comparing the median between the “End of life” groups.

The values in Table 2 (mean, median, maximal distance) are also consistently higher for *P. spumarius* females than for

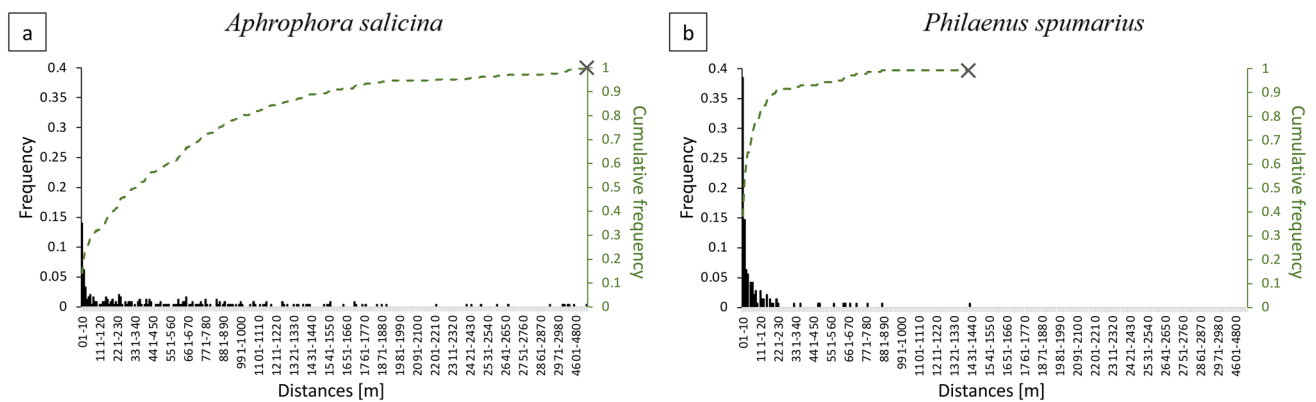


Fig. 4 Frequency of the flight distances travelled in 2.5 h on a flight-mill **a.** for *A. salicina* and **b.** for *P. spumarius*. On the right of the graphs, the y-axis gives the frequency of different distances, which are represented by different bars; on the left, the y-axis gives the

cumulative frequency of different distances observed, which is represented by the cumulative frequency curve, namely, the green dashed line. Graphs generated with Excel 2016



Fig. 5 Boxplot of the distances travelled in 2.5 h on flight-mill by the flying insects according to females' physiological state and sex, expressed in the logarithmic of the distance, for **a.** *A. salicina* and **b.** *P. spumarius*. The light gray boxes represent the distances travelled by the female groups and the dark gray by the male groups. The boxes range from the 25th to the 75th percentile of the distribution values for each group, for which the horizontal line indicate

the median value and the vertical lines the range of values. Different upper-case letters indicate significant differences between the physiological states in the female group and different lower case letters to differences in the male group. * indicates significant differences between the male and the female groups in the same physiological state. Graphs were generated with the open-source software R. © 4.0.4 (R Core Team, 2021)

males in the same physiological state, again with the exception of the "End of life" group. The glm function on the linear model regression revealed significant greater distances covered by females than by males in the "Early adult life" group ($p=0.032$) and in the mating group ($p=0.017$) (Fig. 5). Between different physiological states, the females at the end of life flew significantly less than the females in their early adult life ($p=0.032$), and less than the females in their immature stage ($p=0.017$) and in their mating stage ($p=0.017$). On the other hand, the distances covered by the females at the end of life did not differ from those covered by the egg-laying females. In the case of

males, "immature" group flew significantly greater distances than the "early adult life" group ($p=0.032$), the "mating" group ($p=0.032$) and the "end of life" group ($p=0.032$).

Combining MRR and flight-mill data through modeling

The estimate of daily mean dispersal distance (α ; [m]) based on flight-mill data was 13.09 m for *A. salicina* and 12.42 m for *P. spumarius*, with a negative exponential kernel fitting well the distribution of the distances recorded on

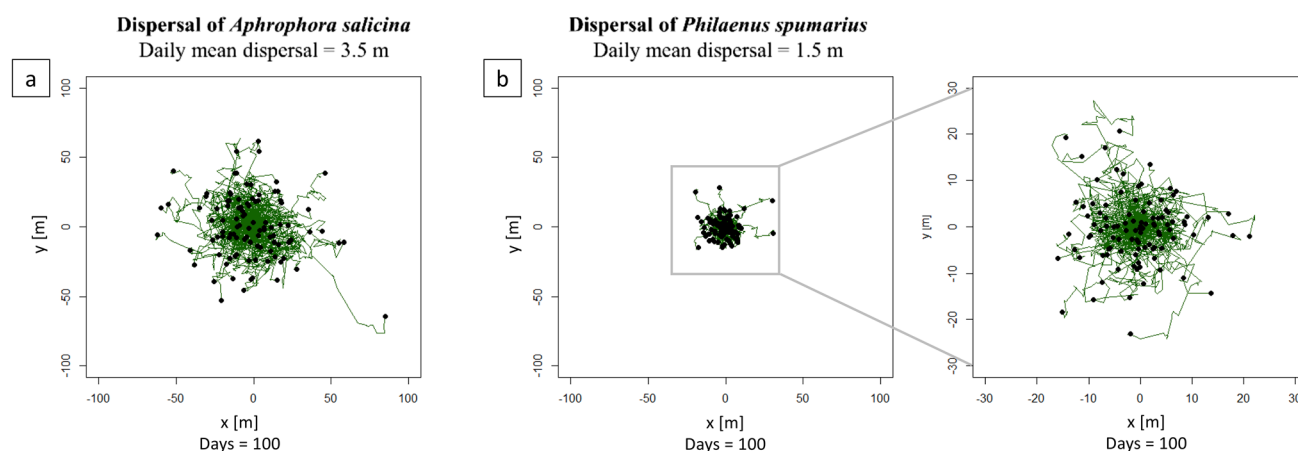


Fig. 6 Simulation of the dispersal of 100 insects on 100 days based on the model of Robinet et al. (2018, 2019). Data were gathered from flight-mill and mark–release–recapture experiments; the parameters introduced were changed with the values corresponding to the ones of *A. salicina* and of *P. spumarius* (probability of flight: 47.9% for *P. spumarius* and 83.3% for *A. salicina*; adult longevity: 153 days for *P. spumarius*, 121 days for *A. salicina*; the number of days needed for

resting after one flight: varying from 0 to 1 day; daily mean dispersal: 1.5 m for *P. spumarius* and 3.5 m for *A. salicina*). The simulation was run using the R software (R Core Team, 2021). **a.** Dispersal for *A. salicina*. **b.** Dispersal for *P. spumarius* with one graph at scale 100 m×100 m and a zoom at scale 30 m×30 m. Graphs generated with the open-source software R[®] 4.0.4 (R Core Team, 2021)

flight-mill. The flight probability, recorded on flight-mills in 2021, was considered 83.3% for *A. salicina* and 47.9% for *P. spumarius*. After fine-tuning the model by selecting the parameters best fitting the MRR data obtained for the year 2020, the obtained value for alpha was 3.5 m for *A. salicina* and 1.5 m for *P. spumarius*. By running the dispersal model 100 times on 100 insects, *A. salicina* were found beyond the 50 m area after 12 days (0.02% of the population, 2/10000 insects). Starting from the 12th day, the number of *A. salicina* found beyond the 50 m area continued to increase until reaching 9% (thus, 900/10000 insects) after 100 days. Depending on the simulations, the first day on which an insect was observed beyond the 50 m could vary (approx. from day 6 to day 16). Even so, according to all simulations, after 100 days, the number of insects beyond the 50 m area always reached the 8–9% of the population. In contrast, no *P. spumarius* was observed beyond the 50-m zone during the 100 days of simulation. A simulation of the dispersal of 100 insects within 100 days is shown in Fig. 6. For *A. salicina*, the average distance after 100 days was around 28 m, depending on the simulation, while the maximal distances were varying between 60 and 100 m. Concerning *P. spumarius*, the average distance was around 9 m after 100 days, while the maximal distances ranged between 20 and 30 m.

Discussion

This is the first study on the dispersal capacities of *A. salicina*, while the flight behavior of *P. spumarius* has been addressed more recently (Bodino et al. 2021; Lago et al.

2021a) due to the recent threatening status of this latter species in Europe, confirmed as vector in Italy, Spain and France (Cornara et al. 2016; Cruaud et al. 2018; López-Mercadal et al. 2021). Here, the dispersal of both species has been investigated through mark–release–recapture and flight-mill experiments.

The poor and great flyers: *P. spumarius* vs. *A. salicina*, potentially driven by host plant distribution

Both experiments revealed that *A. salicina* was significantly a “better” flyer than *P. spumarius*. On flight-mills, almost twice as many *A. salicina* as *P. spumarius* individuals started to fly, and the distances flown were at least 4 to sixfold longer for *A. salicina*. The model combining the data obtained in MRR and flight-mill experiments also estimated that the daily mean dispersal of *A. salicina* was higher than the one of *P. spumarius* (3.5 m vs. 1.5 m, respectively). After the release from the take-off platform, *P. spumarius* moved by jumps while *A. salicina* flew. *Aphrophora salicina*'s first flights could be rather considerable, given that the insects could reach high willow trees (approx. 20 m high) more than 30 m from the releasing point. The zigzag and rotating trajectories possibly indicate that the insects were specifically looking for willows. *Philaenus spumarius*' first movements consisted mainly in 1 m jumps toward the herbaceous layer. The insects often remained in the herbaceous cover without undertaking any other jump for the whole duration of the daily observation. Different behaviors between the

two spittlebug species are likely due to differences in their host plant range. In fact, *A. salicina*, which is closely associated with willows, often needs to fly to reach new trees, while the polyphagous *P. spumarius* does not require to travel far or high to find a suitable host. Our results support the hypothesis of host distribution-driven movement, which has already been inferred in previous studies. Bodino et al. (2021) hypothesized that the differences observed in different MRRs experiments between *P. spumarius* and *N. campestris* could be due to different feeding habits. In fact, *N. campestris* was recovered at more than 2 km from the releasing point in MRR carried out in olive grove in Spain (Lago et al. 2021b) probably because it travelled to reach coniferous plants which are preferred hosts for adults of this species (Morente et al. 2018), while 155 m was the maximal distance recorded for the highly polyphagous *P. spumarius* in olive agroecosystem of Italy (Bodino et al. 2021). However, this difference can also be biased by the sampling protocol as Bodino et al. (2021) focused on the natural dispersal at medium scale (i.e., up to 250 m), while Lago et al. (2021b) sampled insects until a maximum distance of 2.8 km.

Nevertheless, there is other evidence that host plant distribution may account for variability in dispersal between closely related insects or within a given species, including in our study in which the distances covered by *A. salicina* varied between different sites. In fact, *A. salicina* moved farther in the semi-natural site D, which consisted in an open meadow environment surrounded by willows distant from each other by 10 m. The insects were therefore presumably driven to move farther compared to the other sites, where willows were connected to each other in a more closed environment. Similarly, the lower dispersal activity reported for *P. spumarius* in this survey, when compared to previous studies carried out in Mediterranean areas, could be explained by differences in environmental conditions and host plant quality of the study sites. In fact, MRR experiments in Italy allowed Bodino et al. (2021) to calculate dispersal kernels that suggest that, after 30 days, 98% of the released insects would fly up to 283 m (9.4 m/d) in an olive grove in Apulia and 383 m (12.8 m/d) in a meadow in Piemonte, while, in our study, the maximal distance corresponded to 32 m after 27 days. In the Apulian site, such as in other Mediterranean areas, ground vegetation dries during the summer, forcing *P. spumarius* to migrate in order to reach plants with succulent foliage (Cornara et al. 2016; López-Mercadal et al. 2021; Morente et al. 2018). Regarding the Piedmontese pasture, the ground vegetation had dried only in one out of three MRR trials, corresponding to the insects' farther dispersal recorded by the authors (Bodino et al. 2021). In the other two sites, a higher dispersal than in our study could also have occurred due to drier environmental conditions of southern Europe compared to the northern ones, given the high sensitivity of xylem-sap feeding insects to water stresses

affecting their hosts (Krugner and Backus 2014). Change in plant turgor could explain why aestivation to woody hosts in late spring has also been observed in places of southern Europe where the herbaceous layer persists (Bodino et al. 2019, 2021). This may account for the fact that aestivation behavior is not observed in northern regions such as Belgium (authors' personal observation) as the higher moisture allows to maintain an attractive ground vegetation, explaining a more limited *P. spumarius*' dispersal.

Furthermore, studies on *Homalodisca vitripennis* (Germar, 1821), a *X. fastidiosa* vector in California, reported a significantly farther dispersal in abandoned fields characterized by a scarce vegetation, than in citrus orchards (Blackmer et al. 2004; Coviella et al. 2006). Similarly, the latter species showed a higher dispersal in a low-quality host plot (*Prunus persica*) compared to a high-quality host plot (*Lagerstroemia indica*) (Northfield et al. 2009). In summary, our study provided further evidence that the dispersal of a *X. fastidiosa* vector or of potential vectors is likely driven by host plant connectivity and quality, which may change throughout the season (Coviella et al. 2006; Bodino et al. 2021).

Distance over- and under-estimation, different methods possibly balance the bias

Flight-mills are assumed to overestimate the distance travelled by flying insects in wild conditions (Robinet et al. 2019) because it is unlikely that an insect would fly long distances in a straight line and because of inertia possibly maintaining the insect in movement even if it stopped actively to fly (Attisano et al. 2015). In addition, the lack of tarsal contact with the ground may also stimulate the insects to fly longer than under natural conditions where they have the opportunity to land. However, although in some insect species, this lack of contact would force the insect to fly (Edwards 2006; David et al. 2014; Attisano et al. 2015), both species studied had no tarsal reflex since many individuals were observed not moving at all when attached to the flight-mill arm as already observed by Lago et al (2021a) with *P. spumarius*.

When using the flight-mill to study insect flight, a certain level of distress due to handling is also expected. The latter might have either pushed insects to fly farther and longer or weakened them, causing an over- or under-estimation of their flying abilities, respectively. In fact, it was shown in this study that insect manipulation on ice reduced their propensity to fly. To tether insects on a flight-mill, cooling or anesthesia with carbon dioxide can be used (Attisano et al. 2015), both having shown to have a detrimental impact on the insect behavior (Erber 1975; Schumacher et al. 1997). In addition, friction caused by the device can also lead to an underestimation of the flight capacity. On *Agrilus*

planipennis (Fairmaire, 1888; Coleoptera: Buprestidae), Taylor et al. (2010) showed that flight-mills underestimated the flight speed by a factor of 2–3 compared to free flights.

On the other hand, even if MRR experiments estimate the dispersal while considering environmental conditions, they might underestimate the flight abilities of a population, as: the greater the distance, the more difficult it is to recapture insects, due to the increase in surface area and to the dense and complex vegetation. This would explain the shorter travelled distances recorded with MRR compared to flight-mill. In MRR, the decrease over time and the low recapture rates (14.1% after 24 h and 0.9% after 20 days for *A. salicina*; 31.3% after 24 h and 0.6% after 20 days for *P. spumarius*), similar to the rates obtained in other MRR experiments, underline the difficulty of recollecting insects. For instance, in Bodino et al. (2021), the a recapture rate was 0.95% for *P. spumarius* within 17 days (in Italy), while, in Coviella et al. (2006), the rate of recovered *H. vitripennis* was 0.48% within three weeks (in the USA). Although traps have been placed beyond 100 m to overcome this difficulty of recapturing insects, their efficiency in capturing spittlebugs was not sufficient in our trial, as similarly reported in another study (Morente et al. 2018).

While the above-mentioned methods are characterized by their bias, the model that gathered the data from both experiments estimated dispersal distances rather coherent and in accordance with our field observations. In fact, as *P. spumarius* travelled by approximatively 1-m jumps and did not need to travel long distances to find a new host, a mean travelling distance of 1.5 m per day, as estimated by the model, is very plausible. Similarly, a mean daily dispersal of 3.5 m for *A. salicina* seems realistic considering that on average over a day, part of the population would tend to remain on the same tree while another would undertake flights to reach a nearby willow. To conclude, the combination of different methods could therefore possibly balance the different bias. However, it is important to note that this model is isotropic, and therefore, the direction and the absolute distance travelled by an insect from a specific point during the season will be, in natural conditions, influenced by the structure of the environment.

Long dispersal increases the challenge of implementing effective control measures

Based on the dispersal model gathering data from both experiments, the maximal distances reached by the insects in 100 days were between 60 and 100 m for *A. salicina*, and between 20 and 30 m for *P. spumarius*. The EU phytosanitary measures imposed in case of detection of a new *X. fastidiosa* outbreak include the up-rooting of all host plants within a 50 m radius (recently reduced from 100 m) around the detected infected plants (EC, 2020) and were already

highlighted as inefficient by several authors (Bodino et al. 2021; Lago et al. 2021a). Considering our findings, a delimited area of 50 m would probably be enough to encompass all the plants that *P. spumarius* could reach by active flight over a season, as long as the bacterium is promptly detected. However, these measures do not seem to be sufficient to stop potential infection by *A. salicina* through the season if this species proves to be able to transmit the bacterium.

Furthermore, several elements can influence these maximum distances, while flight-mill and MRR trials have shown that both species have the ability of long flights. In fact, the maximal distances on flight-mills performed in 2.5 h were 1.54 km for *P. spumarius* and 6.16 km for *A. salicina*. This last distance represents the maximal value reported in the literature for the Aphrophoridae tested on flight-mills, in that *P. spumarius* travelled 5.5 km in a 5.4 h continuous flight (Lago et al. 2021a), while *N. campestris* performed 1.4 km in 82 min (Lago et al. 2021b). When considering environmental conditions, MRR also revealed potential high flight capacities, with more than 30 m in a single flight performed by *A. salicina* in natural environment, and an absolute distance travelled from the releasing point of 80 m in two days in a site structured with willow corridors. As explained previously, site structure and environmental conditions are both elements that may affect active dispersal and therefore, the probability of long flight. For example, host plant corridors could enhance it, while presence of major roads might decrease it. Similarly, stressful conditions may alter insect movement, increasing or decreasing it, e.g., climate change could decrease the quality of host plants stimulating accordingly spittlebugs' movement, which would fly further to find more nutritious hosts.

Besides external factors, long flight performance may also depend on the gender and physiological state of insects. In fact, in addition of highlighting flight heterogeneity between individuals of the same sex and within the same physiological state, flight-mills also displayed differences between genders and states. The results suggested that females would be more likely to fly further. In other studies, it had already been noted that females show greater dispersal activity than males, such as in the case of *P. spumarius* (Halkka et al. 1967; Lago et al. 2021a) as well as for other froghoppers (Cercopidae) and leafhoppers (Cicadellidae) (Waloff 1973; Nilakhe and Buainain 1977; Lago et al. 2021a). Furthermore, the flying activity of *P. spumarius* females would tend to decrease during the season and the greater distances observed in July at first flight for *A. salicina* in their mating state could also reflect a period of higher flight activity. This would be in line with other studies indicating that immature and mating females are the most active flyers and that egg-loads can lead to a more stationary behavior (Blackmer et al. 2004).

Finally, our surveys and the resulting maximum distances did not take into account passive human-mediated movement

of spittlebugs. As a fact, Aphrophoridae may be hitchhikers, passively moving into vehicles with people or goods, or with plant consignments, influencing the spread of *X. fastidiosa* from infected to bacterium-free areas (EFSA, 2015; White et al. 2017). For example, Strona et al. (2020) showed that even very low probabilities of long-distance dispersal of infected vectors could lead to a dramatic expansion of the outbreak in the closely spaced network of olive groves in Andalusia. They argued that the impossibility to stop long-distance dispersal might compromise the efficacy of other control measures such as plant removal and containment-zone surveillance, and recommended its prioritization over other control strategies when resources are scarce (Strona et al. 2020). While the conditions and the components of potential *Xylella* pathosystem differ between southern and northern temperate Europe, the “if” and “how” the bacterium could establish in northern Europe is widely uncertain (Occhibove et al. 2020). Therefore, even in these northern area, low probabilities of long flights must not be underestimated in the expansion of a potential outbreak.

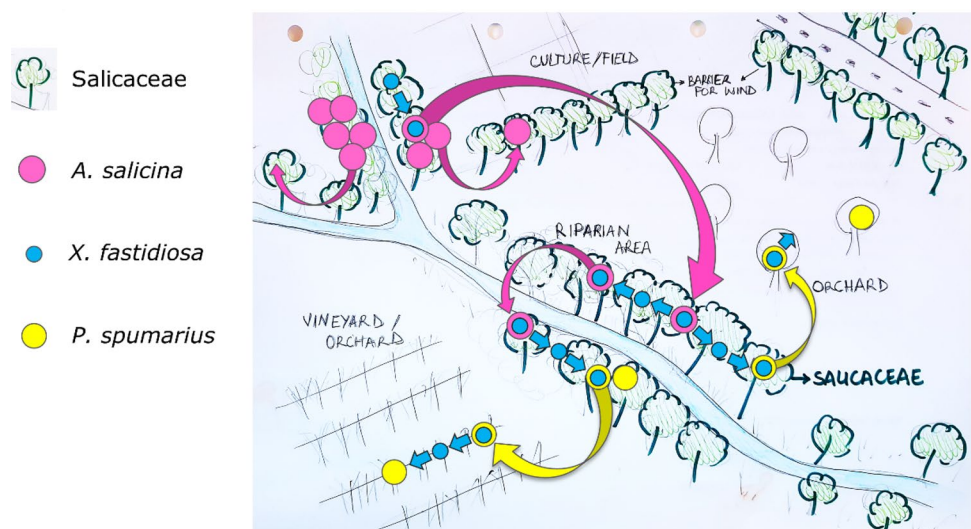
Potential reservoir combining Aphrophoridae and Salicaceae in riparian areas

In northern temperate European areas, *A. salicina* and *P. spumarius* are both species abundantly found in riparian areas (Hasbroucq et al. 2020). These areas are also harboring large densities of Salicaceae plants associated with these insects, for which the widely distributed *S. alba*, *S. caprea* and *P. tremula* have been highlighted as potential host for *X. fastidiosa* through inoculation trials (Casarin et al., submitted; available on BioRxiv); especially, *A. salicina* can be particularly dense on willows in these areas (Hasbroucq et al. 2020). A parallel density study undertaken in two Belgian sites (Supplementary file 3) assessed

that both *S. alba* and *S. caprea* showed *A. salicina*-oviposition marks at 30% of their branches and that on average, an egg-laid shoot generated two adults, suggesting that a tree composed with 1000 branches could potentially carry up to 600 *A. salicina* adults. Although the reported values may of course vary according to sites, they give an idea of the alarming number of insects possibly found on a single tree. Besides riparian areas, Salicaceae are also widely planted in alignments along roadsides and near fields and crops for wind protection, forming a network crisscrossing the landscape. These zones may therefore constitute potential reservoirs where *A. salicina* could be able to spread the bacterium throughout these corridors on medium to long distances. Moreover, *P. spumarius*, reported throughout Belgium colonizing all types of environments (Hasbroucq et al. 2020), has also been observed on Salicaceae in the MRR experiments and has shown to be able to transmit *X. fastidiosa* to *S. alba* in transmission trials performed with infected vectors in Majorca (Casarin et al., submitted). Therefore, although its dispersal appears to be more limited, its polyphagy could lead to a steppingstone effect by spreading the bacterium more locally to other plant species bordering riparian areas, including ornamentals and economically important crops (Fig. 7). Limited movement of vectors near reservoir areas has already resulted in serious outbreaks. For example, in California, riparian areas harbor several vectors feeding on naturally infected host plants, and vineyards adjacent to these zones were already severely affected by Pierce’s disease before the introduction of the efficient disperser *H. vitripennis* into the area (Purcell 1974; Rapicavoli et al. 2018).

Our dispersal data can serve to model on specific areas for contingency plan elaboration. In these more developed models, assessment of the insect density on targeted sites (See Supplementary file 3 for a method for *A. salicina*) and

Fig. 7 Potential *Xylella fastidiosa*-based pathosystem combining Aphrophoridae, Salicaceae and other potential host plants by steppingstone effect. *Aphrophora salicina*, abundant on Salicaceae, can act as long- and medium-distance connector. *Philaenus spumarius*, short connector but polyphagous, could take over on a multitude of plant species, including Salicaceae



considering site structure making the model anisotropic, would provide more accurate estimate of dispersion and maximum distances. However, there are still many data gaps hindering efficient modeling at local or global scale, including the transmission efficiency of the different insect species on selected potential host plants in European temperate areas, such as on Salicaceae. Another important data gap is the exact distribution and abundance of potential host plants in Belgium and northern areas, mainly willows and poplars for *A. salicina*, and how they are interconnected along riparian areas, roads and crops. Due to *P. spumarius*' polyphagy and to the large list of *Xylella* hosts (EFSA, 2022), investigating the potential of other plant species to host the bacterium, in association with candidate vectors, is also essential in order to build up a global view on the potential spread of *X. fastidiosa* in new areas.

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Authors contributions JCG, SH, CB, NC conceived and designed the research. SH, NC, GC, AG conducted dispersal experiments. GC adapted the dispersal model gathering flight-mill and mark–release–recapture data. SH and GC carried out the abundance experiments of *A. salicina*. SH produced the dispersion maps. NC and SH wrote the manuscript. All coauthors commented on previous versions of the manuscript and read and approved the final manuscript.

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Data availability Additional data will be provided by the authors upon reasonable request.

Declarations

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

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Consent to participate Not applicable.

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