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Research

Dispersal of *Philaenus spumarius* (Hemiptera: Aphrophoridae), a Vector of *Xylella fastidiosa*, in Olive Grove and Meadow Agroecosystems

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Abstract

The introduction of the *Xylella fastidiosa* Wells bacterium into Apulia (South Italy) has caused the massive dieback of olive trees, and is threatening olive production throughout the Mediterranean Region. The key vector of *X. fastidiosa* in Europe is the spittlebug *Philaenus spumarius* L. The dispersal capabilities of *P. spumarius* are poorly known, despite being a key parameter for the prediction of the spread of the bacterium. In this study, we have examined the dispersal of *P. spumarius* adults in two different agroecosystems in Italy: an olive grove in Apulia (Southern Italy) and a meadow in Piedmont (Northern Italy). Insects were marked with albumin and released during seven independent trials over 2 yr. The recapture data were pooled separately for each agroecosystem and used to estimate the dispersal kernels of *P. spumarius* in the olive grove and in the meadow. The diffusion coefficient estimate for *P. spumarius* was higher in the meadow than in the olive grove. The median distance from the release point for 1 d of dispersal was 26 m in the olive grove and 35 m in the meadow. On the basis of our model, we estimated that 50% of the spittlebug population remained within 200 m (98% within 400 m) during the 2 mo period of high abundance of the vector on olives in Apulia. The dispersal of *P. spumarius* is thus limited to some hundreds of meters throughout the whole year, although it can be influenced to a great extent by the structure of the agroecosystem.

Key words: spittlebug, mark-release-recapture, dispersal kernel, *Xylella fastidiosa* spread

The dispersal of insect vectors is crucial for plant pathosystems because it affects the spread, prevalence, and incidence of insect-borne plant pathogens to a great extent (Power 1992, Byrne 2008, Krugner et al. 2012a, Strona et al. 2020). Understanding the movement and dispersal patterns of insects, both within and between crops, is thus important to design sound control strategies against vector-borne plant pathogens, and should be taken into account in the planning of pest management programs (Power 1990, Jeger 2000, Weintraub and Beanland 2006, Plantegenest et al. 2007, Sicard et al. 2018).

Plant diseases caused by the xylem-limited bacterium *Xylella fastidiosa* Wells are of major plant health concern worldwide (Almeida 2016, EFSA 2018). The recent introduction of *X. fastidiosa* subsp. *pauca* ST53 into South Italy has led to a dramatic epidemic disease on olive trees, known as Olive Quick Decline Syndrome (OQDS) (Saponari et al. 2014, 2017). *Xylella fastidiosa* is currently spreading north across the Apulia Region at rate of about 20 km/yr, thus threatening the olive production in Italy and in the Mediterranean area (Fierro et al. 2019, Saponari et al. 2019). Since *X. fastidiosa* was identified in the Apulian olive groves, other foci of *X. fastidiosa* strains, belonging to different subspecies, have also been detected on different plant species in southern France (including the Isle of Corsica), Spain (including the Balearic islands), central Italy and northern Portugal (Landa et al. 2020). None of these foci have progressed into such severe epidemics as in Apulia (EFSA 2018, Soubeyrand et al. 2018, Moralejo et al. 2019), although an outbreak

on almonds in Alicante (Spain) led to the demarcation of an infected area of about 140,000 ha (Olmo et al. 2017).

The plant-to-plant transmission of *X. fastidiosa* is only possible through xylem-sap feeding insect vectors, and froghoppers/spittlebugs (Hemiptera: Cercopoidea) and sharpshooter leafhoppers (Hemiptera: Cicadellidae: Cicadellinae) in particular (Redak et al. 2004, Bosco 2015, Cornara et al. 2019). The dominant xylem-sap feeders in Europe are spittlebugs (Hemiptera: Aphrophoridae), unlike the American pathosystems, where sharpshooters are associated with most of the *X. fastidiosa* outbreaks (Redak et al. 2004, Almeida et al. 2005, EFSA 2015, 2019).

The meadow spittlebug *Philaenus spumarius* L. (Hemiptera: Aphrophoridae) is the key vector of *X. fastidiosa* in Europe, where it is widespread and abundant in many habitats (De Jong et al. 2014, EFSA 2015, Cornara et al. 2018). *Philaenus spumarius* is an extremely generalist xylem-sap feeder, feeding on more than 500 species of host-plants, and therefore may transmit the generalist bacterium *X. fastidiosa* to a variety of susceptible cultivated and wild plants (Weaver and King 1954, EFSA 2018, Cornara et al. 2019). The effective control of polyphagous vectors, especially if indigenous, is a challenging task. In Apulia, some agronomic measures—i.e., tilling or mowing—have proved to significantly reduce juvenile populations (EFSA 2018, Di Serio et al. 2019, Dongiovanni et al. 2019, Saponari et al. 2019). However, no satisfactory measures are yet available for the control of the adult stage of spittlebugs (Cornara et al. 2018, Dongiovanni and Fumarola 2018, Dongiovanni et al. 2018, Di Serio et al. 2019). Adult spittlebugs acquire the bacterium by feeding on infected plants soon after emergence in late spring and then, as the bacterium persists in their mouthparts, they can spread the bacterium over the crop during the summer/autumn, mainly through secondary transmission (olive-to-olive) (Cornara et al. 2017b, Morente et al. 2018, Bodino et al. 2019).

Knowledge of the adult stage dispersal capabilities of a long-living vector, such as *P. spumarius,* is fundamental to understand the spread of *X. fastidiosa* across the olive agroecosystem, and to design effective control measures against the adult stage of vectors. Indeed, several studies carried out on American pathosystems, thus involving different vectors (sharpshooters) and crops (grapevine or citrus), suggest that vector mobility is one of the key factors that affect the spread rates of *X. fastidiosa*, together with the population density of the vector and the pathogen transmission rates (Farias et al. 2004, Redak et al. 2004, Daugherty and Almeida 2009, Krugner et al. 2012b).

Despite the importance of vector dispersal on the spread of *X. fastidiosa*, very few data have been published on the dispersal capacity of *P. spumarius* or other Aphrophoridae species. Spittlebug adults are considered poor fliers, as they often just walk or jump instead of flying (Weaver and King 1954, Cornara et al. 2018). Nonetheless, according to Weaver and King (1954), these insects can move up to 300 m in 1 d, and a recent study, using a flight mill, has suggested that they can move up to 1,000 m in a single 1 h flight (Lago et al. 2020). Spittlebugs fly mainly at herbaceous vegetation level (15–70 cm), with only a small proportion found above this level, although a few individuals were collected up to a height of 84 m (Freeman 1945, Weaver and King 1954, Halkka et al. 1971, Reynolds et al. 2017).

Very few of the previous studies attempted to investigate the dispersal of spittlebugs in field conditions using a quantitative approach, such as the mark-release-recapture method (MRR hereafter), which is considered the main method to study the dispersal of insects (Turchin and Thoeny 1993, Hagler and Jackson 2001, Jones et al. 2006). The only MRR study concerning *P. spumarius* reported in literature managed to recapture only one specimen out of 418 marked at about 50–200 m of distance from the release point, and thus was not able to provide any useful information on the dispersal of this species (Halkka et al. 1967). In a recent MRR study carried out in Spain with another spittlebug species, i.e., *Neophilaenus campestris* (Fallén), a few individuals were found at up to 2 km from the release point (Lago et al. 2020).

The aim of this study has been to experimentally estimate the dispersal capabilities of *P. spumarius* within different agroecosystems. We decided to focus on the natural dispersal of vectors at a medium spatial scale (i.e., up to 250 m) in different periods of the year, without considering longer-distance movements toward different habitats, e.g., migration. Indeed, the spread of *X. fastidiosa*, once the bacterium is introduced into a new area, is best predicted by considering local and radial dispersal (Schneider et al. 2020), which is mainly caused by the neighborhood dispersal of its vectors, i.e., movements into and via adjacent areas (Southwood and Henderson 2000). The role of the vectors' longer-distance movements—both natural and human-mediated—in *X. fastidiosa* epidemics still needs to be studied in depth, and Ad hoc studies are therefore necessary (White et al. 2017, Strona et al. 2020). Local dispersal estimates are crucial to 1) model the spread of *X. fastidiosa* from foci in Italy and Europe, which has so far been based on a rough estimate of the vectors' movement (i.e., 100 m) (EFSA 2015), and 2) understand the epidemiology of diseases caused by *X. fastidiosa* under different agroecosystem/landscape conditions.

In this work, we conducted MRR trials to determine the movement capacity of *P. spumarius* adults within two agroecosystems—an olive grove and a meadow—in two Italian regions (Apulia, South Italy and in Piedmont, North Italy) during different periods of the year (May to October). The recapture data obtained in this study are the first quantitative information available on the in-field dispersal of *P. spumarius* adults and they were used to elaborate a spittlebug dispersal model that is able to estimate the distance covered by this insect throughout its adult life, thus providing the rational for *X. fastidiosa* spread containment measures.

Materials and Methods

Experimental Sites

MRR experiments were carried out in two Italian regions (Apulia and Piedmont) under different agro-ecological conditions. One olive grove, under an organic regime management, was selected in the demarcated infected area in Apulia (municipality of Surbo, province of Lecce). The experimental site was thus located in one of the main olive growing areas in Apulia, close to the Adriatic coast at an elevation of 28 m above sea level. The experimental olive orchard consisted of 12-yr old cv Carolea trees, planted at a regular spacing (5 × 6 m), with a somewhat high prevalence of *X. fastidiosa* infection, but still in a good vegetative condition, with canopies suitable for insect refuge and tender shoots for insect feeding. The olive grove was surrounded on the North-East and South-East sides by lentisk (*Pistacia lentiscus* L.) and true myrtle (*Myrtus communis* L.) plants, both of which are typical of Mediterranean vegetation (garrigue) (Fig. 1c and d; see Supp Table S1 [online only] for a detailed description).

The experimental sites in Piedmont were located inside La Mandria Regional Park, near Torino. The natural park hosts the residual of a lowland oak forest, and is characterized by interspersed woods and grasslands, the latter being represented by both semi-natural grassland and unfertilized meadows used for fodder production (Sindaco et al. 2008). Two different meadows,

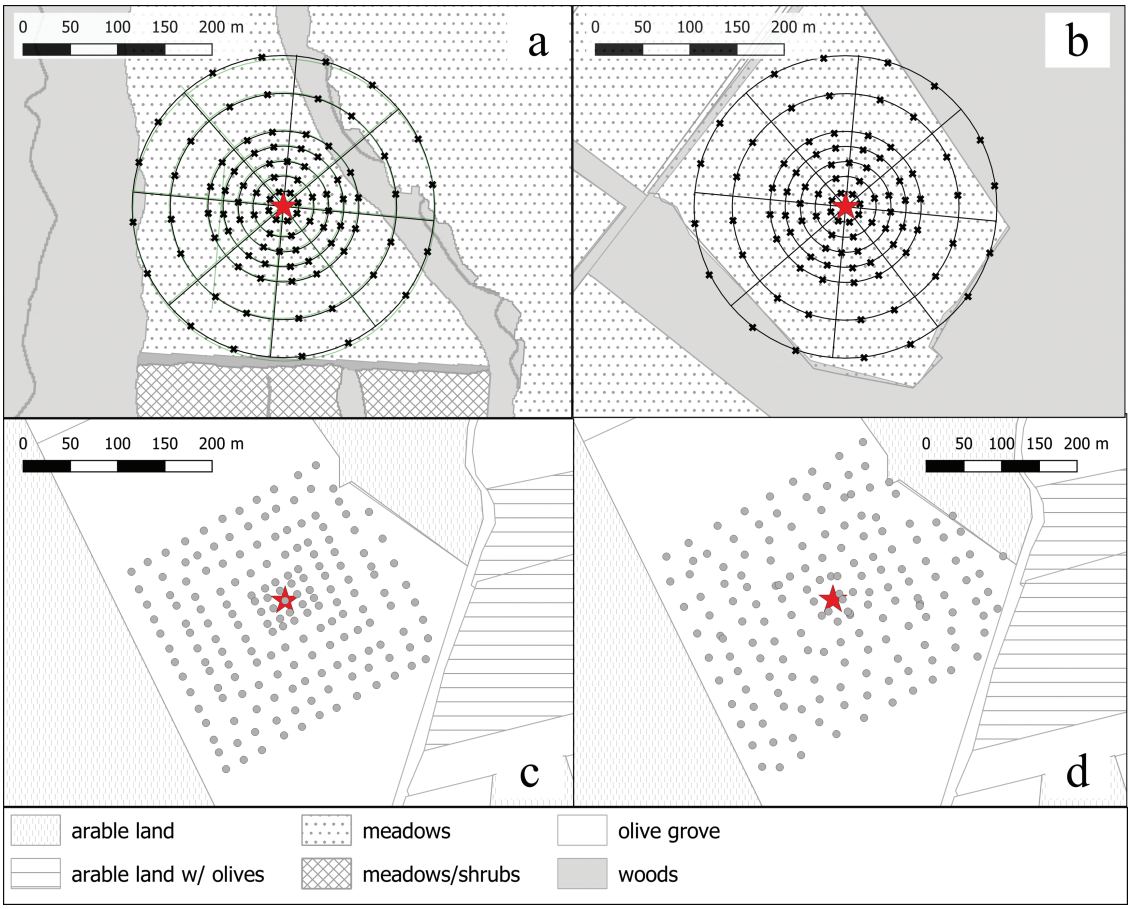

Fig. 1. Location of the MRR experimental sites and schematic layout of the sampling points in Piedmont (a: 2016, b: 2017) and Apulia (c: 2016, d: 2017). Star shapes represent release points of *P. spumarius* adults in Piedmont and Apulia sites.

surrounded by woods and other grasslands, were selected for the experimental assays in 2016 and 2017 (Fig. 1a and b; see Supp. Table S1 [online only] for a detailed description). No mowing or other farming practices were applied at the experimental site during the sampling campaigns. Some of the external recapture points were located at the border of the meadow/wood or within the wood nearby; sweeps were also carried out on the wild woody plants (*Quercus robur* L., *Acer campestre* L., *Carpinus betulus* L., *Robus* spp., *Crataegus monogyna* Jacq.) that were present in these recapture areas.

Insect Preparation

Capturing the Insects

Philaenus spumarius adults were collected from the herbaceous cover of the organic meadows (untreated, grass cut once-twice per year), within a distance of 50 km from the experimental sites (*X. fastidiosa*-free areas) in both Regions. Sweeping nets and manual aspirators were used in order to capture the insects alive. The collected insects were placed in plastic cages (≈ 25 × 25 × 25 cm [l × h × w]) with two mesh sides, together with cut forbs for nutrition, and were then placed in a cooler bag and brought to the laboratory facilities in Bari and Torino. Field collections were carried out 2 to 5 d before the start of the MRR experiments, in order to limit the time spent by the insects in the high-density rearing environments, i.e., insect-proof cages (2 × 2 × 1.5 m [l × w × h]). Rearing cages were placed outside the laboratory facilities under a shading net. These cages contained a mixture of different potted plants, i.e., *Polygala myrtifolia* L., grapevine, *Erigeron* spp. (Apulia), or at least 10 pots with 2–3 broad bean plants each (≈ 3wk old, BBCH 14–15) (Piedmont).

Marking the Insects

Albumin (pasteurized chicken egg whites: Eurovo SRL, Calisese di Cesena, province of Forlì-Cesena, Italy) was used as a marker. Albumin is frequently used in MRR experiments, as it is inexpensive and more detectable than other markers (Jones et al. 2006, Hagler 2019). Preliminary experiments, carried out in the laboratories in both Torino and Bari, were conducted to test for the persistence of albumin on marked insects, even after exposure to artificial rain. Albumin was used as deionized water solution (10%) with no wetting agents added (Lessio et al. 2014). A manual sprayer was used to directly apply a fine mist of albumin solution onto the spittlebugs and plants inside the rearing cage. The albumin solution was applied twice on two consecutive days on both the insects and plants.

Releasing the Insects

Olive Grove (Apulia)

Cages with the albumin-marked insects were transferred directly to the selected olive grove and—prior to the release of the insects—inspected to estimate the rate of mortality. The cages were then opened under a single olive tree located in the center of the plot (Fig. 1c and d), and the potted plants hosting the insects on the foliage were gently placed onto the main branches, thereby allowing the spittlebugs to spontaneously move away from the potted plants. Five experiments were performed from spring to autumn, in

Table 1. Releases and recapture layout in the Apulia and Piedmont MRR experiments in 2016 and 2017

Region	Year	Release date	Sampled strata	No. of released Spittlebugs	Recapture samplings (days after release)	No. of samplings per date[a]
Apulia						
	2016	04 July 2016	Olive canopies, shrubs	1,000	7, 11, 17	160 (132), 200 (172), 200 (172)
	2017	29 May 2017	Olive canopies, shrubs	1,500	2, 5, 7, 9, 12, 17	177 (149), 176 (148), 324 (296), 325 (297), 324 (296), 361 (333)
		21 July 2017	Olive canopies, shrubs	1,100	3, 5, 7, 10, 12, 17	325 (297), 323 (295), 323 (295), 322 (294), 322 (294), 371 (343)
		04 Oct. 2017	Olive canopies, shrubs, weeds	1,600	2, 5, 7, 9, 12, 14	325 (297), 323 (295), 322 (294), 322 (294), 322 (294), 371 (343)
Piedmont						
	2016	07 Sept. 2016	Ground vegetation	700	2, 5, 12, 15	92
	2017	16 June 2017	Ground vegetation	693	3, 5, 7, 10, 14, 17	92
		13 Sept. 2017	Ground vegetation	1,692	2, 5, 7, 9, 12, 14	92

[a]The numbers outside the parenthesis represent the total number of samplings performed on all the sampled strata per date, while the numbers in parenthesis represent the samplings only performed on olive canopies per date.

the 2016–2017 period (Table 1). The temperature and wind speed were monitored, throughout the experiments, from a weather station (Rete Agrometeorologica della Regione Puglia: Code 0PU11) located within a distance of 4 km from the experimental site.

Meadow (Piedmont)

Insects were collected from the rearing cages 1 d after the last albumin spraying, by means of the manual aspirator, and placed in plastic cages with 2 mesh sides together with cut forbs for nutrition, and were then placed in a cooler bag and brought to the experimental location. Cages containing spittlebugs were placed at the soil level at the release point situated in the center of the experimental site (Fig. 1a and b), with the opening point upwards. The cages were left open for about 15–20 min to allow the insects to spontaneously leave, and they were then gently shaken to free the spittlebugs remaining inside the cages. This protocol was adopted to limit the 'escape effect' caused by the initial handling. Temperature, wind speed, and wind direction data were recorded throughout the experiments from a weather station (Sistema Piemonte: Venaria http://www.sistemapiemonte.it/agricoltura/banca_dati_agrometeo/index.shtml; ARPA Piemonte: Code S4587) located within a distance of 2 km from the experimental site.

In both regions, the releases began in the late afternoon, between 4:00 and 7:00 pm, in order to avoid manipulating the insects during the mid-day hours and thus to limit insect mortality. The release dates, the numbers of spittlebugs released, and recapture days for each trial are reported in Table 1. Low mortality rates were observed for the insects in the release cages (approx. 10–20 individuals/trial, i.e., ≈ 1–2% of the total insects released).

Recapture Layout

The spittlebugs were recaptured on different post-release dates using sweep nets at fixed collection points, corresponding to 1) olive canopies, shrubs and—when present — ground vegetation in Apulia and, 2) ground vegetation in Piedmont. In both cases, the insects were sampled using a sweep net (38 cm in diameter with a 0.8 m long stick), and 10 sweeps were conducted for each sampling point; the whole tree canopy or about 3 m^2 of the ground vegetation surface was swept. Recapturing through sweeping was preferred over other recapture methods, e.g., sticky traps, which are less efficient in collecting spittlebugs (Morente et al. 2018). Moreover, insects collected by means of sweep nets are of a higher quality for the subsequent analysis (devoid of glue components and not exposed to sun or rain degradation), and the collection can be ascribed to a precise time, unlike those captured with sticky traps.

The recapture layouts between the olive grove and meadow differed.

- Sampling design in the olive grove of Apulia region
  Collection points were located at a distance of 10 to 120 m from the release point. Olive canopies represented the majority of sampling points. However, shrubs surrounding the olive grove (lentisk, true myrtle, Rubus spp.) were also sampled to a distance of up to 250 m from the release point. The ground vegetation was only sampled in the autumn experiment in 2017, as it was present as a result of abundant rainfall. From 160 to 200 points were sampled at each sampling date in the 2016 experiment. The collection points were organized in concentric rectangles at different distances from the release point (Fig. 1c). From 176 to 371 points were sampled on each sampling date in the 2017 experiment (Table 1). The collection points were organized in a grid pattern surrounding the central release point, according to the spacing of the olive trees (Fig. 1d). Additional samplings were carried out on wild shrubs surrounding the olive grove, up to a distance of 250 m from the release point, that is, on lentisk (26 sampling points) and true myrtle (2 sampling points). On the last sampling date in the 1st trial in 2017 (Spring), another 37 olive trees were sampled in the area close to the release point: all the olive trees within a 10 m radius from the release point were sampled, as were half of the trees at a distance of 15 m from the release point. On the last sampling date in autumn 2017, the weeds under each olive tree, in correspondence to the sampling points, were also swept for spittlebugs.

- Sampling design in the meadow of Piedmont region
  A total of 92 collection points were located on herbaceous plants at a distance of 10 to 200 m from the release point in a radial pattern (Fig. 1a and b). Recaptures were conducted every 2 or 3 d up to 17 d after the release (Table 1). Some of the collection points were located outside the experimental meadow, within the surrounding woods, thus both the herbaceous cover and woody plants present in these points were sampled. Out of the 92 collection points, 11 were located inside the woods in the 2016 trial, and seven in the 2017 trial.

The *Philaenus spumarius* adults collected in the sweeps were individually transferred into 50 ml tubes, and placed in a cooler bag.

They were then freeze killed and stored at –20°C until the analysis for the presence of albumin was conducted.

Analysis—Marker Detection

An Indirect Enzyme-linked immunosorbent assay (ELISA) was carried out on each individual to detect the presence of the marker on the insect body (Crowther 2001; adapted from Jones et al., 2006). A primary antibody for chicken egg albumin (C6534; Sigma–Aldrich, St. Louis, MO) was used for the assay. The secondary antibody used was goat anti-rabbit IgG (H + L) with alkaline phosphatase (A3687, Sigma–Aldrich). All the incubations were performed at 37°C. Individual insects were transferred to 1.5 ml tubes containing 1 ml TBS (pH 8.0; T-664, Sigma–Aldrich), to which 0.3 g/liter ethylenediaminetetraacetate (EDTA; E5134, Sigma–Aldrich) was added. After 3 min of thorough mixing, by means of vortexing, an aliquot of 80 µl from each tube was loaded into individual wells of a 96-well microplate (Nunc Polysorp; Nalge Nunc, Naperville, IL), with three replicates per sample. The plate was incubated for 2 h, prior to being washed five times with 300 µl PBST (pH 7.4, 8 g/liter NaCl, 0.2 g/liter KCl, 1.44 g/liter Na_2HPO_4; S3014, P9541, S9763, Sigma–Aldrich, respectively) + 0.09% Triton-X100 (T8787, Sigma–Aldrich). The wells were then loaded with 300 µl of blocking solution (PBSS-BS 20%), prepared by diluting (1:5) bovine serum with 520 ppm Silwet L-77 (Helena Chemical, Memphis, TN) in PBS. After an incubation of 1 h, the wells were washed twice with 300 µl PBST. The primary antibody solution was diluted (1:20,000) in 20% PBSS-BS and 80 µl was added to each well. The plate was incubated for 30 min, and then washed five times with 300 µl PBST. The secondary antibody solution was diluted (1:8,000) in 30% PBSS-BS (364 ppm of Silwet L-77) and 80 µl was added to the wells. The secondary antibody incubation lasted 2 h. After incubation, the secondary antibody solution was discarded and the wells were washed five times with 300 µl of PBST. An aliquot of 80 µl of a p-nitrophenyl phosphate (pNPP) substrate (P4744, Sigma Aldrich) was then added to each well, and the plate was placed on a rotary shaker and incubated in the dark at room temperature for at least 10min prior to starting the reading of the absorbance (optical density [OD]), which was conducted at 450 nm using an automatic plate reader. In each plate six negative controls, i.e., unmarked *P. spumarius*, and a positive control, i.e., diluition of albumin (1 µl/ml), were also included. All the readings were corrected (blanked) using wells with a TBS + EDTA extraction buffer and no antigen present. Background cutoff was set at a mean value of negatives + 4 × SD of the negatives.

In preliminary trials, albumin was detected by ELISA on all the marked *P. spumarius* adults for up to 20 d in semi-field conditions (at least six insects were analyzed 2, 7, 15, and 20 d post-treatment, fresh and defrosted). The assay was able to detect a positive signal in as little as 6 ng/ml of albumin from chicken egg white (Sigma–Aldrich) (data not shown).

Statistical Analysis

According to a Brownian motion assumption (Turchin 1998, Nathan et al. 2012), the dispersal capacity of *P. spumarius* can be modeled through a Gaussian dispersal kernel (k_D), that is, a probability density function that represents the probability of a dispersal endpoint at time t at a radial distance r from the point of release:

$$k_D(r,t) = \frac{r}{2Dt} e^{\frac{-r^2}{4Dt}}, r \in [0,+\infty), t \in [0,+\infty),$$

where D is the diffusion coefficient. We adopted the method proposed by Bancroft and Smith (2005), based on Turchin (1998) and Kareiva (1982, 1983), which involves four analytical steps, to estimate the dispersal kernel. In the first step, we computed the mean square displacement (MSD) at each sampling time t_i

$$MSD_i = \frac{1}{N_i}\sum_{j=1}^{N_i}(r_{j,i})^2$$

where $r_{j,i}$ is the distance at which the $j-th$ marked insect was recaptured on the $i-th$ sampling day and N_i is the number of marked insects recaptured on that day. In the second step, the diffusion coefficient for each sampling day $\hat{D}(t_i)$ was estimated as follows:

$$\hat{D}(t_i) = \frac{MSD_i}{4*t_i}$$

Subsequently, the overall diffusion coefficient was calculated as the average of $\hat{D}(t_i)$ over the total number of sampling days of the experiment (M):

$$D = \frac{1}{M}\sum_{i=1}^{M}\hat{D}(t_i)$$

In the last step, the estimated parameter D was used to parametrize the dispersal kernel. The results are described by considering the median distance from the release point at time t, $R_{50}(t)$, and the 98th percentile of the diffusion model:

$$R_{50}(t) = \sqrt{4*D*t}$$

$$R_{98}(t) = 2*\sqrt{4*D*t}$$

where $R_{50}(t)$ corresponds to the radius of the circle centered at the release point which encompasses 50% of the population of released insects after$t$ days from the release. The circle of radius $R_{98}(t)$, centered in the release point, encompasses 98% of the population of released insects after$t$ days from the release.

Results

Recaptures

Approximately 8,300 meadow spittlebugs were marked and released in seven different experimental trials performed over the 2 yr in the olive grove and meadow agroecosystems. Details of the number of *P. spumarius* (marked or resident, i.e., non-marked) captured in each experiment, and sampling date, are shown in Table 2. A graphical description of the distances at which the marked insects were collected is provided in Figs. 2 and 3. Overall, the recapture rate of the released spittlebugs was low (0.95%), with a total of 79 marked *P. spumarius* adults being recollected. The recapture rate was lower in the olive grove (0.67%) than in the meadow (1.43%), possibly due to the sampling of different vegetation types—canopies in the olive grove in Apulia and a herbaceous cover in the Piedmont meadow. Sweep sampling on canopies is usually less efficient (Bodino et al. 2019) than on herbaceous vegetation. Most of the recaptures occurred at sampling points near the release point, e.g., 50% of the recaptured individuals were sampled within 30 m from the release point in Apulia, and within 40 m in Piedmont. However, a few spittlebugs were recaptured after a few days from the release at the furthest sampling distance in the recapture grid, i.e., 120 m (olive canopy) and 155 m (herbaceous cover) in Apulia (Fig. 2), and 200 m (meadow) in Piedmont (Fig. 3).

The samplings in Apulia were mainly carried out on olive trees, and therefore most of the marked insects were recaptured on olive

canopies (28 out of a total 35: 80%). However, when weeds were present, because of the autumn rain, during the third trial in 2017, 7 out of 10 marked insects were sampled on the herbaceous cover (see triangles in Fig. 2). No insects were recaptured on the wild plants bordering the olive grove.

Climate/Seasonal Effects

It was not possible to relate any clear differences in dispersal capabilities to the climatic data registered in the nearby meteorological stations. The mean temperatures and rainfall were different between the summer and autumn experiments in Apulia, with the June–July period being notably hotter and drier than October, that is, the usual climatic condition in the Mediterranean area (Supp Table S2 [online only]). The more abundant precipitations in September–October allowed a new herbaceous cover to grow, which attracted the spittlebugs after the dry summer period, as can be seen from the recapture data (triangles in Fig. 2). The wind speed and direction were only recorded in the 2016 trial, and no clear preferential dispersal direction was observed in the spittlebug recaptures. The mean temperatures and wind speed were not significantly different for the three experimental trials in the Piedmont sites (Supp Table S2 [online only]). The wind direction was predominantly North or South, and no clear preferential dispersal direction was observed in the spittlebug recaptures (Fig. 3). Marked individuals were recaptured at longer distances from the release point during the 3rd trial in the Piedmont meadow, i.e., six individuals recaptured ≥ 150 m, that is, 43% of the total recaptures, compared to the previous two trials when only one individual was recaptured at a farther distance than 100 m (Fig. 3). Late summer in the Piedmont site, i.e., August and September 2017 (3rd trial period + the previous month), was drier (20 mm cumulative rainfall) than the previous two trial periods (167–210 mm cumulative rainfall). However, given the low recapture number, no statistical analysis was performed on these data.

Dispersal Kernel

The diffusive behavior of *P. spumarius* was studied under two very different agroecosystem conditions, i.e., an olive orchard and a meadow. Thus, we estimated the dispersal kernel in the olive orchard and in the meadow separately, pooling together the trials performed in the same agroecosystem. The estimates of the diffusion coefficients, the median and the 98-percentile distances (expressed in meters) are shown in Table 3 considering five time steps: 1, 30, 60, and 90 d after the release, and at the end of the dispersal period of the *P. spumarius* adults (day 210). According to previous studies (Di Serio et al., 2019; Bodino et al., 2019), we considered an average adult life duration of 210 d, during which *P. spumarius* can disperse. The 30–60–90 d time steps in Table 3 represent approximations of the time periods of the year during which the abundance of *P. spumarius* on olive canopies is high. These periods of high density of spittlebugs on olives are generally shorter in Apulian olive groves (May-June, ≈ 30–60 d) than in Ligurian ones (North-Western Italy, late May–August, ≈ 90 d) (Bodino et al. 2019, 2020). The dispersal kernels estimated for the two agroecosystems are shown in Fig. 4. The curves represent the probability density function of the distances from the release point where insects can be found after 30, 60, 90, 120, and 210 d from the release. The curve of the 1-d time step after release is not shown for the sake of clarity.

In the olive orchard, the estimated dispersal distance of 50% of the population was at most 141 m from the release points 1 mo after the release (98% of the population is within 283 m)

Table 2. Number of marked and resident (in parenthesis) *Philaenus spumarius* captured during the MRR trials in Apulia and Piedmont

Region/trial	Days from release												Overall	
	2	3	4	5	7	9	10	11	12	14	15	17	Captures	No. of samplings
Apulia														
July 2016	—	—	—	—	4 (30)	—	—	1 (37)	—	—	—	0 (32)	5 (99)	560
June 2017	0 (81)	—	—	1 (57)	2 (121)	1 (147)	—	—	3 (148)	—	—	8 (126)	15 (680)	1,687
July 2017	—	2 (105)	—	1 (63)	1 (49)	—	0 (34)	—	0 (31)	—	—	1 (27)	5 (309)	1,986
Oct. 2017	1 (32)	—	—	1 (33)	1 (31)	3 (25)	—	—	2 (30)	2 (40)	—	—	10 (191)	1,985
Piedmont														
Sept. 2016	3 (99)	—	—	7 (153)	—	—	—	—	0 (208)	—	3 (208)	—	13 (668)	368
June 2017	—	3 (36)	—	4 (24)	2 (33)	—	2 (30)	—	—	3 (27)	—	1 (28)	15 (178)	552
Sept. 2017	6 (55)	—	—	4 (45)	1 (45)	1 (56)	—	—	3 (55)	1 (54)	—	—	16 (310)	552

Dash indicates days when no samplings were performed during the trial.

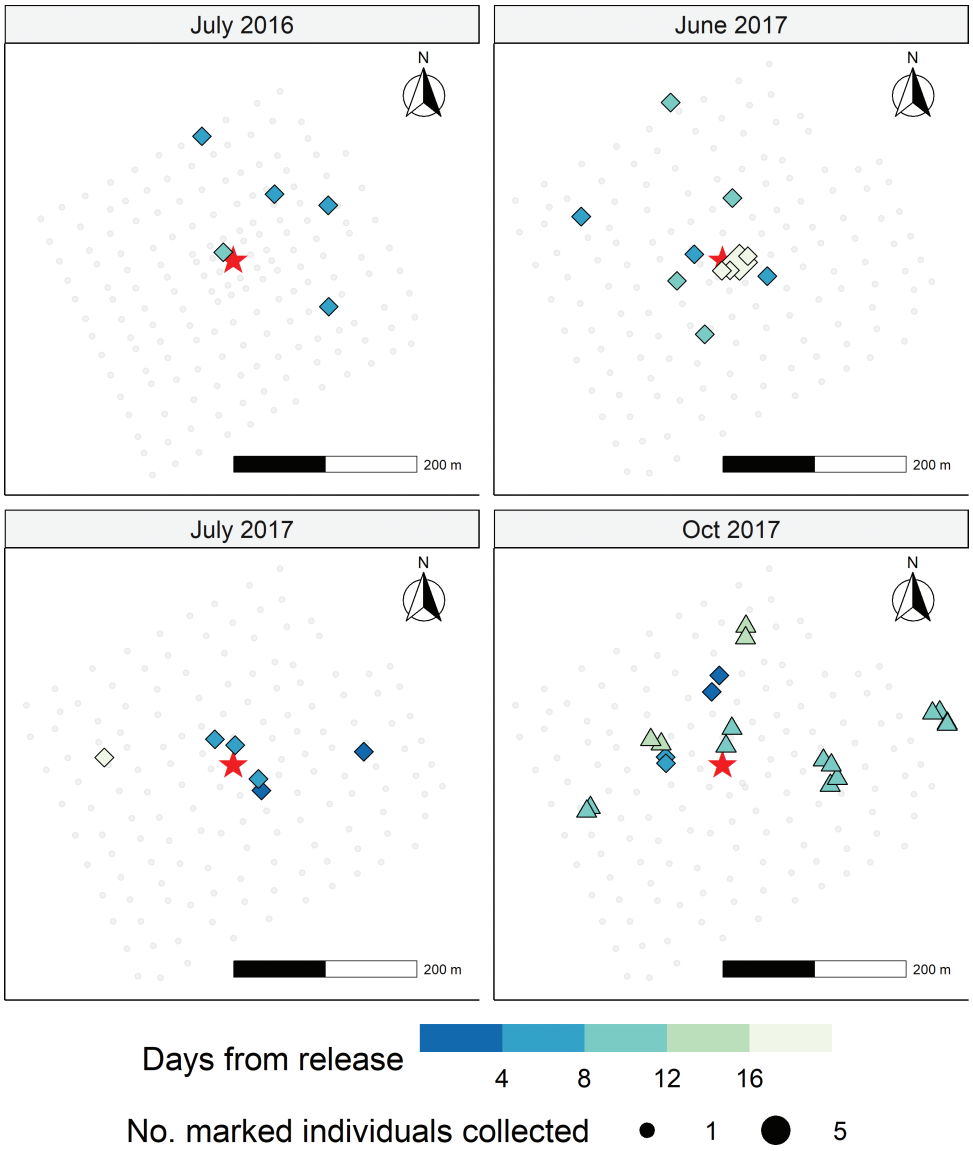

Fig. 2. Recaptures at different times post-release of marked *Philaenus spumarius* adults at fixed recapture points surrounding the release point in the Apulia experimental trials. Diamond and triangle shapes represent recaptures obtained from olive canopy and herbaceous plants, respectively. Star shapes represent release points. A very small amount of variation of recapture points position was included to make visible multiple recaptures obtained at different times at the same sampling point.

(Table 3). Ninety days after release, 50% of the population dispersed no farther than 245 m from the release point. On day 210, the dispersal kernel had significantly flattened (Fig. 4a), that is, the population was dispersed over a wider area, R_{50}(210)was equal to 374 m and 2% of the population dispersed over 748 m from the release point (Table 3). A higher diffusion coefficient has been estimated from the experimental data for the meadow agroecosystem. In fact, 1 mo after the release, the estimated dispersal distance of 50% of the population was at most 192 m from the release point (98% of the population was within 383 m) (Fig. 4b). After 210 d from the release, 50% of the population had dispersed over 507 m from the release point and 2% of the insects had spread over 1 km from the release point. On the basis of the dispersal kernel, we estimated that 50% of the population of *P. spumarius* adults had spread over 100 m from the release point, i.e., the current limit for testing and removing *X. fastidiosa* host-plants around positive plants, 15 d after the release in the olive grove and 8 d in the meadow.

Discussion

This work is the first experimental study on the dispersal capability of *P. spumarius*, the main *X. fastidiosa* vector in Europe. The dispersal capacity of insect vectors is a key parameter in determining the spread of insect-borne plant diseases (Purcell et al. 1985, Zhou et al. 2002, Orenstein et al. 2003, Krugner et al. 2012b, Shaw et al. 2017, Sicard et al. 2018). As far as *X. fastidiosa* epidemics in Europe are concerned, the current EU mandatory measures require the removal of all plants susceptible to *X. fastidiosa* within a radius of 100 m around the plants tested positive to the bacterium ('EU 2015/789' 2015, EFSA 2018). The 100 m estimate was based on a few previous descriptive studies on *P. spumarius*, e.g., by Weaver and King (1954) and Halkka et al. (1967),

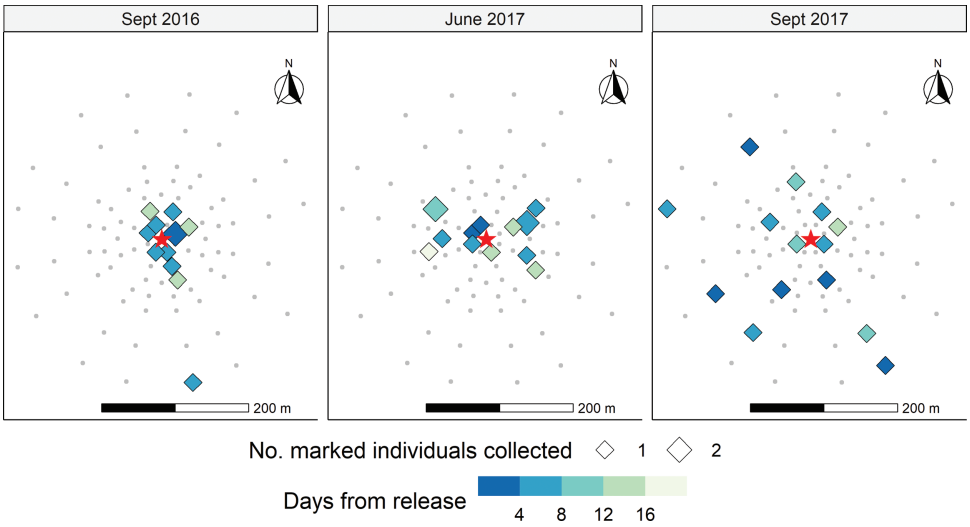

Fig. 3. Recaptures (diamond shapes) at different times post-release of marked *Philaenus spumarius* adults performed at fixed recapture points on herbaceous plants surrounding the release point in the Piedmont experimental trials.

and on experiments carried out on sharpshooters in the United States in different agroecosystems (citrus orchards) as well as ones under threat in Europe (mainly olive groves) (Blackmer et al. 2006, Northfield et al. 2009).

Our study indicates that *P. spumarius* may disperse even farther than 100 m during their adult life. The recapture rate was generally low, especially in the Apulia olive grove, even though the marking protein (i.e., albumin) was quite persistent in both the preliminary and experimental trials, and marked insects were recaptured up to 17 d after release. The spittlebugs were mostly recaptured in proximity of the release point (20–60 m) throughout the experiments, even after 2 wk from release. However, a few individuals were able to disperse even farther to a distance of up 200 m in the meadow within 2–5 d from release. The distribution of the dispersal distances, estimated by means of a diffusion model adapted from Turchin (1998), shows that *P. spumarius* adults tend to disperse over time from a single release point, and they dispersed for longer distances in the meadow than in the olive grove agroecosystem. Although the daily median dispersal capacity was limited (i.e., 26 m in the olive grove and 35 m in the meadow), we estimated that half of the population could disperse even farther than 370 m in the olive grove (Apulia) and farther than 500 m in the meadow agroecosystem (Piedmont) during their entire adult life (estimated as 210 d). Since the transmission of *X. fastidiosa* in Apulia is mostly due to secondary spread, that is, from olive-to-olive (Cornara et al. 2017a), the expected median dispersal rate of infectious insect vectors can be taken as a proxy of the spread of *X. fastidiosa*. This estimate should also take into account the colonization time of olive canopies by *P. spumarius*. Indeed, vector adults are not present on olive trees throughout their entire adult life, and in the Apulian olive groves, where the period spent by *P. spumarius* on olive canopies is quite limited (May–June, up to early July) (Bodino et al. 2019, 2020), the expected median dispersal rate of infectious insect vectors among olive trees is about 200 m. On the contrary, in North Mediterranean olive groves (e.g., the Liguria Region of Italy), spittlebugs stay longer on olive trees (about 3 mo, June–August) (Bodino et al. 2019). Thus, the estimated median dispersal distance of *P. spumarius* among olive trees in those conditions would be higher, i.e., 245 m. According to our model, half of the *P. spumarius* population was able to disperse in the olive grove at farther distances than 100 m in 15 d.

Most of the prior studies on the movement of spittlebugs have been observational, and only rarely experimental, and none of them attempted to model the dispersal capabilities of these insects, thus a direct comparison with our dispersal estimates is difficult. For example, massive migrations of *P. spumarius* in Ontario, as reported by Putman (1953), have only been observed a few times, and this spittlebug species is considered exotic in North America, where it can reach much higher densities than those reported in Europe; thus it is possible that the dispersal capacity is influenced positively by such extremely high densities (Mundinger 1946, Everly 1959, Wiegert 1964). Weaver and King (1954) reported *P. spumarius* adults moving distances of up to 100 m from the release point within 24 h, and during an MRR study performed in Finland, only one spittlebug was recaptured at ≈100 m a few days after release (Halkka et al. 1967).

A recent study carried out in Spain on the dispersal of *N. campestris*—another European spittlebug species—has highlighted the flying capabilities of this species, which is able to disperse for long distances in a mixed agricultural landscape (300 m in 8 d and up to 2 km in 35 d) (Lago et al. 2020). The high dispersal capabilities observed for *N. campestris* by Lago et al. (2020) is only apparently in contrast with our estimates for *P. spumarius,* as several factors could have affected their results: 1) the influence of the smaller size of *N. campestris*, compared to *P. spumarius*, on both the active fly capabilities and wind dispersal (Angelibert and Giani 2003, Jenkins et al. 2007); 2) the migration behavior, which is different from the dispersal behavior and is characterized by an escape movement to abandon a habitat (Schneider 1962). Since their study was conducted in a summer period, when the ground vegetation has dried out under the Mediterranean climate, the insects were forced to move to patches with woody plants in order to have succulent foliage for aestivation (Drosopoulos et al. 2010); 3) the host-plant association of *N. campestris* adults, which mainly feed on coniferous plants (especially *Pinus* spp.) (Lopes et al. 2014, Morente et al. 2018). For this reason, *N. campestris* adults are compelled to move farther to reach patches with these plants, whereas *P. spumarius* adults are polyphagous and, during the summer period, are associated with

Table 3. Estimates of the dispersal coefficient and the median or 98%-th percentile of the dispersal kernel distribution for Philaenus spumarius adults in the olive grove and meadow agroecosystems for five time steps

	Sampling site			
	Olive orchard (Apulia)		Meadow (Piedmont)	
n^a	35		44	
D^b	166.3		306.1	
Time *t* (day)	median	98%	median	98%
1	26	52	35	70
30	141	283	192	383
60	200	400	271	542
90	245	489	332	664
210	374	748	507	1014

the presence of several woody plants in the Mediterranean garrigue, especially *Quercus* spp. and *Pistacia* spp. (Bodino et al. 2020). Furthermore, Lago et al. (2020) did not consider the distribution of the proportion of the marked population over different distances from the release points, thus it is also possible that the individuals recaptured at 2 km from the release point were a very small portion of the population mainly dispersed at closer distances from the release point. In fact, 80% of the recaptured insects were found at 130–280 m from the release point, thus on average having moved 3.6–6.5 m/d.

Our dispersal estimates of *P. spumarius* adults are also quite similar to those observed and modeled for some species of sharpshooters in American agroecosystems. *Homalodisca vitripennis* (Germar), the main *X. fastidiosa* vector species in South California, can disperse to up to 150 m in 3 d in citrus orchards (median dispersal rate = 31 m) (Blackmer et al. 2006), although some studies have reported lower dispersal rates, e.g., 10–20 m median dispersal in 25 d in a *Prunus persica* orchard (Northfield et al. 2009) or up to 20 m from the release point in a citrus orchard (Coviella et al. 2006). Interestingly, *H. vitripennis* can move significantly farther in abandoned fields, with scarce vegetation, that is, more than 155 m in 6 h (Blackmer et al. 2004), and at least 100 m within minutes (Coviella et al. 2006), than sharpshooters released in citrus orchards (Blackmer et al. 2004, Northfield et al. 2009). A similar behavior was observed for the *Deois flavopicta* (Stål) (Hemiptera: Cercopidae) spittlebug in Brazil, which is able to fly for hundreds of meters on unfavorable patches (plowed fields), but likely to stay close to the release point in optimal patches (pastures), e.g., 80% of the released insects were recaptured within 2 m from the release point after 24 h (Nilakhe and Buainain 1988).

The actual dispersal of insects under natural conditions is usually associated more with the propensity to move than to their flight performances (Steyn et al. 2016, Gray et al. 2020). The dispersal propensity of spittlebug adults cannot be intended as fixed, but varies as a result of several factors, both external (environment) and internal (intrinsic status of the organism) (Steyn et al. 2016). Among the external factors that can influence their dispersal, the landscape composition plays a major role; both the distribution of wild plants in olive groves and the mosaic of close surrounding agroecosystems determine the spatial distribution and movement of vectors (Santoiemma et al. 2019, Bodino et al. 2020). Moreover, the patch quality and period of the year also seem to be essential components that affect spittlebug dispersal (Bodino et al. 2019). These two aspects are often correlated, since a patch or agroecosystem that is optimal in a certain period may become sub-optimal in other periods (e.g., due to drying of the ground cover) (Cornara et al. 2017b, Morente et al. 2018, Bodino et al. 2020). Xylem-sap feeding insects are highly sensitive to both plant turgor and the nutrient composition of xylem (Brodbeck et al. 1990, 2011; Andersen et al. 2005). Therefore, seasonal changes in the host-plant physiology can influence the dispersal of insects to a great extent, especially during the summer period in a Mediterranean climate, when spittlebugs tend to move from agroecosystems to wild woody plants (Mizell et al. 2008, Drosopoulos et al. 2010, Bodino et al. 2020). In our study, the meadow agroecosystem showed longer dispersal estimates, and these results could be related to the sub-optimal quality of the herbaceous vegetation. We registered the highest dispersal rates in the meadow trial during autumn 2017, when the ground cover was dry, as a result of the absence of rainfall in the experimental location during late summer. Moreover, the wind speed and prevalent direction can also determine the dispersal of spittlebugs at farther distances, as these insects may be collected at a high altitude, although with a very low frequency (Freeman 1945, Reynolds et al. 2017). Nonetheless, spittlebugs mainly move at a low altitude (i.e., <1 m) (Weaver and King 1954, Nilakhe and Buainain 1988) and, due to their relatively heavy body weight, are unlikely to be efficiently transported by airflows or passively transported by wind. Temperatures can also influence insect movements and their propensity to fly, e.g., the flying activity of Cicadellidae is usually inhibited by low temperatures (Waloff 1973, Larsen and Whalon 1988, Coviella et al. 2006). Although we did not specifically test the effect of wind and temperature in our dispersal experiments, the climatic data registered in weather station close to the experimental locations did not show any clear effects on spittlebug dispersal.

Among the internal factors, the physiological development and age of the insect may also affect the dispersal of spittlebugs, in particular, egg maturation in late summer and oviposition on herbaceous cover in late summer-autumn (Weaver 1951, Witsack 1973, Di Serio et al. 2019). However, given the low recapture rate, we did not observe any differences in the dispersal of *P. spumarius* adults for different periods of the year or between sexes. Moreover, the spittlebug movement dynamics at a landscape level is still largely unknown, though it is known to influence the abundance of insects and the presence of bacterium reservoirs outside crops (Cruaud et al. 2018, Santoiemma et al. 2019, Lago et al. 2020). Future studies should be aimed at improving the recapture rate of marked spittlebugs, possibly using both sweeps (efficient and precise but a time-consuming method) and sticky traps (less efficient and precise but which allow the number of both recapture points and recaptured insects to be augmented) (Morente et al. 2018).

In short, our work provides sound estimates of the dispersal capabilities of *P. spumarius* adults, the main vector of *X. fastidiosa* in Europe, in both olive grove and meadow agroecosystems in Italy. We estimated a higher dispersal rate in the open agroecosystem (meadow) than in the tree-dominated agroecosystem (olive grove). Our results suggest that 50% of the *P. spumarius* population that visits olives moves up to a distance of 200 m and only 2% moves farther than 400 m during the 2 mo population peak period on the crop in Apulia

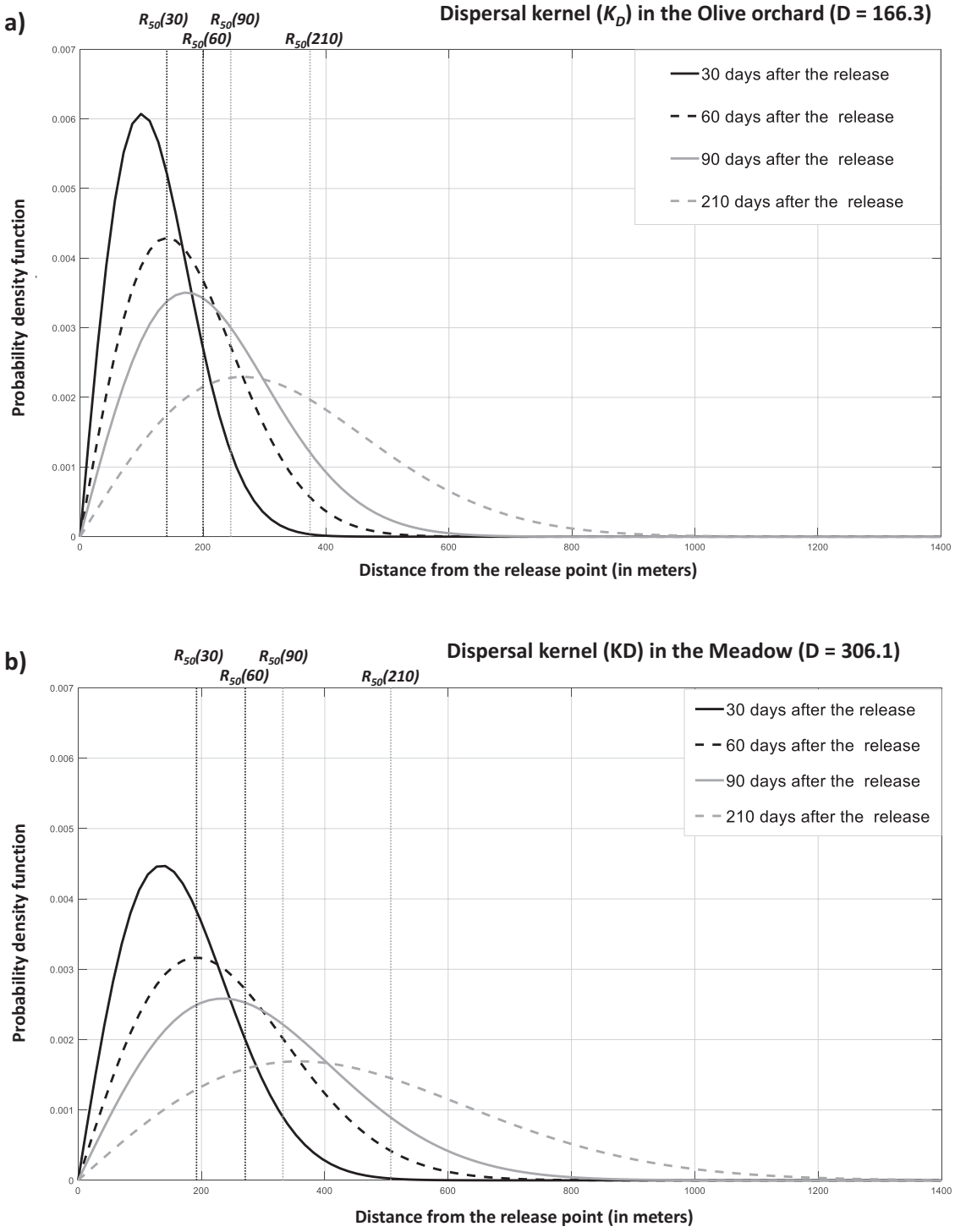

Fig. 4. The dispersal kernels of *P. spumarius* in a) the olive grove (Apulia) and b) the meadow (Piedmont). The curves represent the probability density functions of the dispersal distance of the in-sect populations 30, 60, 90 and 210 days after release. The vertical dotted lines represent the median distances (R_{50}), i.e. the radius of the circle centered in the release point encompassing 50% of the population, 30, 60, 90 and 210 days after release.

(May–June). Further studies should address the effect of factors that could influence both the magnitude and directionality of dispersal (e.g., patch type, food resources, host-plant physiology, climatic conditions, control measures) in order to understand the dispersal drivers of vectors both within and among agroecosystems. Containment measures of the epidemic spread in South Italy and in other European foci should consider dispersal estimates to elaborate effective strategies in order to limit the spread of this quarantine bacterium.

Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

Acknowledgments

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