Effects of different abiotic and biotic factors on spatial primary seed dispersal in the semachorous species *Scrophularia canina*

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Abstract

Seed dispersal is one of the most important steps in the plant life cycle. However, there is, generally, a lack of fieldworks focused on wind dispersal and especially on semachorous (seeds spread when the fruits are shaken by wind and other vectors, such as animals), including boleochorous species. Therefore, we aimed to determine how different types of wind and animals affected seed dispersal under natural conditions in the widespread species *Scrophularia canina*. We evaluated the effects of wind gusts (simulating them using a leaf blower) and wild animals (using differently sized dogs) on seed dispersal in a population located in south-western Europe. We found that *S. canina* is a semachorous species, and its spatial seed dispersal was affected by wind gust speed and direction, plant structure, and vector type. The results also revealed the presence of xerochasy, individual anisotropy with strong winds, and primary short-distance dispersal associated with successional processes independent of the vector. Additionally, there was a masking effect of plant structure on the seed shadow outline. It is essential to conduct fieldworks to reveal what actually happens in nature, taking into account the characteristics determining seed dispersal. In addition, in these works it is important to find out what factors affect seed distributions of anemochorous and semachorous species.

Keywords: anisotropy, boleochory, leeward and windward dispersal, semachory, xerochasy.

INTRODUCTION

Seed dispersal is one of the most important steps in the plant life cycle because it influences the spatial structure and dynamics of plants at population and metapopulation levels. Seed dispersal determines the distribution of individuals, affects the probability of incorporation of new individuals into a population, and the possibility to establish new populations and connect existing populations, i.e., the distribution of individuals within and across populations (Maier, Emig, & Leins, 1999; Greene & Calogeropoulos, 2002; Soons & Bullock, 2008; Brzosko et al., 2017 and references therein; Jordano, 2017).

The seed shadow represents the distances reached by diaspores. Ecologically and in terms of distance, seed dispersal has been traditionally classified into two categories: short-distance and long-distance dispersal (henceforth SDD and LDD, respectively). A genetic viewpoint has been established by determining the simultaneous demographic and genetic effects of seed dispersal. This viewpoint is necessary for predicting the response of individuals, populations, and species to climate change and ecosystem fragmentation (Trakhtenbrot, Nathan, Perry, & Richardson, 2005; Robledo-Arnuncio, Klein, Muller-Landau, & Santamaría, 2014; Jordano, 2017). Local SDD would ensure the maintenance of populations (but see Jordano, 2017), while LDD maintains connectivity among populations, allowing gene flow, but having homogenizing effects (Brzosko et al., 2017; Jordano, 2017). In addition, LDD contributes to the establishment of new populations (Maier, Emig, & Leins, 1999; Thomson et al., 2010; McConkey et al., 2012; Brzosko et al., 2017; Jordano, 2017) and could determine the survival of a species, especially in fragmented landscapes (Primack & Miao, 1992; Maier, Emig, & Leins, 1999; Vittoz & Engler, 2007; McConkey et al., 2012).

The maternal plant and dispersal vector can significantly influence the dispersal distance. Maternal plant traits include plant architecture, height, and lateral spread (Bastida & Talavera, 2002; López-Vila & García-Fayos, 2005; Rodríguez-Riaño et al., 2017 and references therein), fruit dehiscence type and fruit position (Kadereit & Leins, 1988; Blattner & Kadereit, 1991; Bastida & Talavera, 2002; López-Vila & García-Fayos, 2005; Rodríguez-Riaño et al., 2017), and diaspore adaptations to dispersal (Jongejans & Telenius, 2001; Bullock, Moy, Coulson, & Clarke, 2003; Vittoz & Engler, 2007). Nevertheless, the most influential factor is the dispersal vector (Bullock, Moy, Coulson, & Clarke, 2003; Vittoz & Engler, 2007; Soons & Bullock, 2008; Savage, Borger, & Renton, 2014).

In anemochorous species, including boleochorous species, the factors that strongly influence the seed shadow are seed properties, wind characteristics (intensity, direction, and turbulence), relative humidity (RH) and plant traits. However, such information is rarely taken into account, because most of the data are based on mathematical models or are collected in wind tunnel experiments, i.e., not under natural conditions (Kadereit & Leins, 1988; Ozinga, Bekker, Schaminée, & van Groenendael, 2004; Soons & Bullock, 2008; Brzosko et al., 2017; among others). Terms like boleochory and semachory could be considered synonymous, but Bonn et al. (2000) clarified that for the latter, in addition to wind, other vectors, such as animals, may shake rigid fruiting branches as well, resulting in seed dispersal.

A few studies on boleochorous (maybe also semachorous) species have been developed under natural conditions (Rodríguez-Riaño et al., 2017), but most have obtained data under semi-natural or artificial conditions (Kadereit & Leins, 1988; Blattner & Kadereit, 1991; Maier, Emig, & Leins, 1999). Besides, these and other papers (Bullock & Moy, 2004) consider them as anemochorous species without taking into account the other vectors that could provoke seed release.

In the plant wind dispersal, it is the wind gusts that exceed the threshold required to cause seed release, and not the average wind speed, that determines non-random seed release and increases LDD (Greene, 2005; Skarpaas, Auhl, & Shea, 2006; Soons & Bullock, 2008; Savage, Borger, & Renton, 2014; Treep et al., 2018). This non-random seed release must be a general phenomenon in boleochorous species, because their fruiting branches must vibrate to release seeds, and this only occurs selectively during rigorous environmental conditions, such as strong winds. In addition, this vibration can be also performed by the rubbing of animals (Bonn et al., 2000), a phenomenon that has not been addressed in the majority, if not in all, of the works carried out using these kinds of plants. In terms of humidity, xerochasy is a widespread phenomenon in wind-dispersed species. In such species, seed abscission and release occur mainly under low RH and high wind speed (Greene, 2005; Schippers & Jongejans, 2005).

Considering the limited information available on boleochorous and semachorous species, and especially, when considering animals as vectors, the main aims of this study were to determine, in a natural population, the effect of both abiotic (wind) and biotic (animals) factors on spatial primary seed dispersal. We analyzed the seed dispersal of the widespread plant *Scrophularia canina* L. (Scrophulariaceae): (i) under different wind gust speeds; (ii) with windward and leeward prevailing winds; and (iii) by differently sized animals.

MATERIALS AND METHODS

Plant and study area

Scrophularia canina, considered as a pioneer, is a suffruticose species that flowers from March to June, with seed dispersal occurring from (September)-October onwards, and sometimes even overlapping with the next seed dispersal period (Rodríguez-Riaño et al., 2017). The fruits are persistent bicarpellate capsules (3–7 mm), slightly acuminate, and contain an average of 9.7 blackish seeds (0.6–1.7 mm) (Ortega-Olivencia & Devesa, 1993) which are dispersed boleochorously (Rodríguez-Riaño et al., 2017, but see discussion below).

The experimental site was situated in south-western Europe (Badajoz Province, Extremadura, Spain) between 313 and 320 m a.s.l. along the road from Albuquerque to Villar del Rey (39°11′6.20″N–6°56′14.83″W). The population was situated between a crop area and a new road, at a site previously occupied by an old road. A line of holm oaks and a small watercourse establish the limit with the crop area (Supporting information Figure S1). The vegetation between the road and the watercourse was a scrub dominated by *S. canina* and scattered individuals of *Quercus rotundifolia* and *Retama sphaerocarpa* (López et al., 2016).

The average wind speed during the previous and current year of the study period was about 8 km/h (range of previous year = 6–9 km/h, range of current year = 5–9 km/h), and the maximum wind speeds were about 37 and 35 km/h, respectively (range = 32–42 and 26–48 km/h, respectively). In the previous and current years, the wind gusts reached to 63 km/h (mean = 49 and 52.8 km/h, respectively) (Weather Underground, 2018).

Field experiments and experimental design

The fieldwork was carried out on 16 individuals from November 2017 until February 2018. The individuals were sufficiently isolated from neighboring plants to ensure accurate identification of diaspore origin.

To evaluate seed fall, we used the same trap model described previously (Rodríguez-Riaño et al., 2017), but extended its length to 300 cm, with one end of the trap situated at the base of the plant stem and labelled as 0 cm. Each trap was a 5-cmwide tape that was stapled sticky side up to a 100-300 x 10 cm fibreboard (see Figure 1). To allow adequate rotation of experimental treatments among and within plant individuals (see below), each individual was divided in four quadrants (coinciding with the four principal cardinal points). The maximum height at the center of each plant and lateral spread (i.e., zone beneath the plant's branches) was measured for each quadrant.

The experiments to study the effect of different vectors on spatial primary seed dispersal included: (1) control, (2) abiotic vectors, and (3) biotic vectors. In the control and biotic experiments, one seed trap was situated in one of the four quadrants (Figure 1b, c). In the abiotic experiments, two seed traps were placed in opposite quadrants (Figure 1a). In the control and abiotic experiments, the plants were subjected to each treatment for 10 min. In the biotic experiments, for those 10 min, the animals were rubbed against the individual three times simulating the randomness of animal contact in nature. Once the experiment ended, the trap was collected, labelled, and transported to the laboratory. For this transport, seed traps were covered with additional tape to prevent seed loss. Seed numbers were counted by using a stereo-microscope. After dividing each tape sample into 1-cm intervals using superimposed, transparent millimeter paper, seed number per cm and maximum distance reached by a seed were determined with the plant stem as distance cero (Rodríguez-Riaño et al., 2017).

Control: A seed trap was placed under the fruiting branches of one of the four quadrants in which each plant was divided. The plant was not subjected to any manipulation.

Abiotic factor experiments: We tested the ability of wind gusts at two different speeds to shake the fruiting branches. Wind was simulated using a leaf blower and its speed was measured with a digital anemometer. In these experiments, two sticky traps were situated under the individual at a maximum of 300 cm from the base of the main stem of the plant: one in the quadrant between the blower and the plant (windward trap, i.e., subjected to upwind) and the other behind the plant (leeward trap, subjected to downwind) (see Figure 1a). Taking into consideration the wind speeds and gust values in the population studied (see above in plant and study area), we applied the following treatments:

Slightly strong wind (simulation with wind gusts up to 30–35 km/h – henceforth ANE-30*)*. In this case, to check 30-35 km/h wind speed the leaf blower was set to a minimum revolution and placing the anemometer close to the blower air outlet, then we moved away until the anemometer reached approximately 30-35 km/h speed. At that moment, we measured the distance from the researcher's waist carrying the blower to the position of the anemometer (1.80 m). Once the blower is turned on and placed 1.80 m away from the centre of the plant individual (reached speed of 30-35 km/h), it was moved from top to bottom and from right to left, during ten minutes, to shake the plant branches for provoking the seed release from the fruits. The trap placed in front of the blower (windward) was labelled as WW (henceforth ANE-30-WW) and that behind the plant (leeward) as LW (henceforth ANE-30-LW).

Extremely strong wind (simulation with wind gusts up to 60–70 km/h – henceforth ANE-60). To check 60-70 km/h wind gusts, the researcher with the leaf blower was situated 2 m away from the anemometer, at this moment we accelerated it until we got the desired speed (60-70 km/h) and fixed the blower at this acceleration. Then, the procedure was the same as that indicated for the 30-35 km/h design. The trap in front of plant was labelled as ANE-60-WW and that behind the plant as ANE-60-LW).

Biotic factors experiments: In these experiments, the shaking of fruiting branches by wild animals was simulated by rubbing differently sized dogs against the plant. For this, once a seed trap was placed under one of the quadrants of the plant, a dog guided by a researcher was forced to rub against the fruiting branches situated in this plant quadrant (Figures 1b, c). This process was repeated three times for the same seed trap during a 10 minutes treatment period. There were two treatments:

Big wild animal (henceforth ZOO-BIG). Fruiting branches were rubbed with a dog about 30 kg (Figure 1b).

Small wild animal (henceforth ZOO-SMALL). Fruiting branches were rubbed with a dog about 3 kg (Figure 1c).

All the above mentioned treatments (control, biotic and abiotic) were replicated six times, and all individuals were subjected to all treatments in each of the replicates. For *Scrophularia canina*, seeds are sequentially liberated from the placenta and gradually released from the fruit. Additionally, the seed shadow is correlated with plant lateral spread (Rodríguez-Riaño et al., 2017). Therefore, we tried to avoid underestimating seed dispersal in the experiments by conducting the different tests in each replicate at least 2 days apart. We also tried to avoid the effect of plant lateral spread by changing the quadrant in which the treatment occurred. In addition, we tried to avoid or minimize the RH effect on seed dispersal by changing the time of the day when each experiment was performed. Thus, all the treatments were implemented from early in the morning (normally with high RH) to late in the afternoon (usually with low RH). Therefore, all the experiments were performed at different times of the day. RH was measured using a thermo-hygrometer at the beginning and at the end of the treatments. In the following we show an example of one replicate:

First day in each replicate: half of the individuals were assigned to: (1) the plant's Eastern control quadrant; (2) one abiotic treatment (for example, wind at 30–35 km/h) at the North and South quadrants; (3) one biotic treatment (for example, big dog) at the plant's Western quadrant. The other half of the individuals were subjected to the other two treatments (60–70 km/h wind speed and small dog) but carried out at different plant quadrants.

Second day in each replicate: each individual was subjected to the treatments it did not receive on the first day.

Spatial primary seed dispersal

To evaluate the dispersal capacity and seed distribution patterns (seed shadow) in each treatment, we determined parameters as described by Rodríguez-Riaño et al. (2017), namely: (1) slope of the linear regression of seed number (ln-transformed); (2) modal dispersal distance (distance reached by the greatest number of dispersed seeds); (3) percentiles of seed dispersal distance (25th, 50th, 75th, 90th, and 99th); (4) maximum dispersal distance (maximum distance reached by a seed); (5) percentage of seeds underneath the plant's lateral spread; and (6) kurtosis and skewness indices.

Seed dispersal parameters were compared among all treatments and with natural seed dispersal (data from Rodríguez-Riaño et al., 2017, 2018). The data were treated in two different ways for these comparisons. Firstly, data for all individuals were merged and used to calculate total seed shadow parameters. Secondly, the parameter mean values were calculated for each treatment and individual, and then these were compared statistically. Differences in parameters among the different treatments were analyzed using the non-parametric Kruskal-Wallis test (more than two independent variables) or Mann-Whitney test (two independent variables) with False Discovery Rate (FDR) adjustment of p-values. The correlations between seed dispersal parameters and plant traits were determined by Spearman's linear correlation analyses.

We calculated the total number of seeds collected in traps in each treatment for each individual. These data were normalized by Log(seed number) and compared using one-way ANOVA. This variable was not compared with natural dispersal because different methods were used.

Finally, considering the merged data, a hierarchical clustering analysis was used to classify the six dispersal treatments and natural dispersal, and a dendrogram was constructed using Ward's method based on Euclidean distance squared.

All statistical analyses were carried out using the SPSS statistical package (IBM Corp., 2013).

RESULTS

In the control, no seeds were dispersed in any of the replicates, so these data were not included in statistical analyses. Wind speed during the control treatment development was at most about 10 km/h, no exceeding, therefore, the threshold needed to cause seed release.

The total number of seeds dispersed during a treatment, taking into consideration the date on which the replicates were carried out, were characterized by a succession of peaks and troughs, which were more apparent in anemochorous than zoochorous treatments and, within the anemochorous, in those with higher wind speed (Figure 2). The successions of the peaks and troughs depended largely on the RH and on the date on which the replicate was performed. Relating to RH, fewer seeds were dispersed during treatments in the early hours of the day (higher RH) than during treatments later in the day (lower RH). For example, on December 1st, the treatments with fewest dispersed seeds were those with higher wind speeds (60 km, both WW and LW), which were performed when the RH was higher (early hours of the morning, about 10:00 am; GMT + 1). More seeds were dispersed when these treatments were conducted again at about noon on December 18th, when the RH was much lower. The effect of RH was very evident on December 21st, which was an intense foggy day. Considering the date of the replicate, the last replicates (from January 14th) usually dispersed fewer seeds than those performed earlier, indicating that the greater the number of seeds in the fruit, the greater the number of seeds dispersed.

Spatial primary dispersal patterns

We could differentiate four different dispersal patterns taking into account seed shadows (Figure 3) and semi-ln plots (Figure 4) obtained using the merged data, namely:

(i) windward anemochory, (ii) leeward 30 km/h anemochory, (iii) leeward 60 km/h anemochory, and (iv) zoochory. To these patterns, we added (v) natural dispersal.

In all seed shadows, two zones could be distinguished: zone 1, from the plant stem to its lateral spread, which is the area harboring most of the dispersed seeds (hereafter, body); and zone 2, from the plant lateral spread to the maximum distance reached by a seed (hereafter, tail).

Natural dispersal: The natural seed dispersal produced a typical leptokurtic seed shadow (Figure 3), with a very pronounced decrease in the first quarter of the body, weakening this decrease as we move towards to its lateral spread, and very few seeds reaching the tail (Figure 3). The semi-ln plot perfectly fitted both linear and quadratic functions (R^2 about 0.94; Figure 4). This seed shadow had the highest slope (−0.044; Figure 4). The body harbored almost 90% of the dispersed seeds (see Figure 5a). All the percentiles were reached at shorter distances than in other treatments (Figure 5b), and the maximum dispersal distance did not exceed 100 cm (Figures 3–5).

Windward anemochory: In both the ANE-30-WW and ANE-60-WW treatments there were a pronounced decrease in seed abundance moving outwards from the plant stem. This decrease weakened with increasing distance from the stem, and there was even a small increase in seed density near the plant edge (Figure 3). The maximum dispersal distance did not exceed 150 cm in ANE-30-WW, and was shorter in ANE-60-WW (about 130 cm) (Figures 3–5). The ANE-30-WW semi-ln plot fitted both linear and quadratic functions (R^2 about 0.84; Figure 4), but that of ANE-60-WW fitted slightly better to a quadratic function ($R^2 = 0.88$) than a linear function ($R^2 = 0.78$). Both treatments had a similar slope (about −0.029) (Figure 4). The body harbored about 80% of the dispersed seeds (Figure 5a). These dispersal patterns had the next lowest percentiles after the natural dispersal pattern (Figure 5b).

Leeward 30 km/h anemochory: There was a continuous and pronounced decrease in seed abundance, stronger close to the plant stem (Figure 3). The tail started with a slight increase in seed percentage compared with the final part of the body, and from this point there was a gradual decrease in seed percentage up to 150 cm from the plant stem (Figure 3). The semi-ln plot fitted both linear and quadratic functions (R^2 about 0.78; Figure 4). The slope was about −0.018 (Figure 4). The body harbored about 65% of the dispersed seeds (Figure 5a). After the ANE-60-LW treatment, this treatment had the second highest percentile values above the 50th percentile. The maximum seed dispersal distance did not exceed 150 cm (Figures 3–5).

Leeward 60 Km/h anemochory: Among all the anemochorous treatments, this treatment showed the largest deviations (Figure 3). The body was almost a flat line, except for a very small increase in seed percentages close to the plant stem (Figure 3). This treatment had the longest tail, with the seeds reaching up to 250 cm. The semi-ln plot, similar to that of 60-WW, fitted slightly better to a quadratic function ($R^2 = 0.81$) than to a linear function ($R^2 = 0.72$) (Figure 4). The slope was about −0.015 (see Figure 4). The body harbored only about 43% of the dispersed seeds (Figure 5a). This treatment had the highest values in all percentiles and the maximum seed dispersal distance (Figures 3–5).

Zoochory: Of all the dispersal treatments, the zoochorous treatments resulted in the largest deviations (Figure 3). The outline was very different to those of the anemochorous treatments, because the seed percentage tended to increase from the stem to the plant edge, with the highest values close to the plant edge (Figure 3). The tail was shorter than those in other treatments, but similar to natural dispersal (Figure 3). Semi-ln plots of both treatments fitted much better to a quadratic function ($R^2 = 0.85$ for big animals and $R^2 = 0.52$ for small animals; Figure 4) than to a linear function (0.46 and 0.35, respectively) (Figure 4). The slopes were very low, about –0.02 for big animals and −0.014 for small animals. The body harbored about 75% of the dispersed seeds (Figure 5a). Among all the treatments, the zoochorous treatments had the highest values for modal distance (about 45–48 cm, *vs*. <5 cm in other treatments) (Figure 5a).

Comparative analysis of different treatments

The statistical analyses of all the seed shadow parameters produced treatment groupings very similar to those obtained from analyses of merged data. Notably, there were large deviations in most parameters, especially modal distance (Figure 7).

The most deviant dispersal treatment was the ANE-60-LW, in which most of its parameters significantly differed from those in other treatments (Figures 6, 7). This treatment had the highest percentiles values (Figure 6). It also had the highest modal distance (Figure 7), but this was not significantly different from those of the zoochorous treatments because of its high standard deviation. By contrast, the global value of ANE-60-LW was only 4 cm (see Figure 5b), similar to those of the other anemochorous treatments (about 1 cm). This treatment had the lowest seed percentage under the plant canopy (43.35% ± 11.86%).

Most of the parameters of the zoochorous treatments were statistically similar to those of the windward anemochorous treatments (Figures 6, 7), although it should be noted that there were large variations in most of the studied parameters (Figures 6, 7).

The parameters of the windward-anemochorous treatments were statistically similar to each other, but the skewness and kurtosis indices were different. The ANE-30-LW treatment was statistically similar to the windward anemochorous and zoochorous treatments in terms of the lower percentiles (25th and 50th), and to windward anemochorous treatments in terms of modal distance (for more details see Figures 6, 7).

Natural dispersal showed statistical similarities with windward anemochorous and zoochorous treatments (for more details see Figures 6, 7) for those parameters farthest to the plant stem (99th percentile and maximum distance reached by a seed, Figure 6), and seed percentage under the plant canopy (Figure 7).

Plant height and lateral spread were not significantly correlated with most of the seed shadow parameters. The anemochorous 60 km/h treatments had the largest number of parameters significantly correlated with plant features (Supporting information Table S1): leeward test parameters (percentiles and skewness index) were correlated with plant height, and windward test parameters (lower percentiles, seed percentage under plant canopy, and skewness and kurtosis indices) were correlated with plant lateral spread (Supporting information Table S1). In the other treatments, few parameters were correlated with plant features (Supporting information Table S1).

A clustering analysis of all the studied parameters classified the dispersal treatments into three groups (Figure 8a). The first group included only the ANE-60-LW treatment. The second group comprised the other anemochorous treatments, recognizing two dispersal patterns: windward tests and the ANE-30-LW treatment. The third group included the two zoochorous tests and natural seed dispersal.

The third group seemed to be very artificial on the basis of their seed shadows (Figures 3, 4). We eliminated all the studied parameters one by one from the clustering analysis, and found that removal of the maximum dispersal distance resulted in different groupings (Figure 8b). In that case, the cladogram grouped the treatments similarly regardless of whether pooled data or data for each individual were used. Group 1 included the leeward treatments; group 2 contained two subgroups: windward treatments and natural seed dispersal. Group 3 included zoochorous treatments. The removal of the 50th percentile similarly changed the clustering analysis (see Figure 8c). The relationships among treatments were very similar in all cladograms obtained. The most drastic change was the relationship between natural seed dispersal and the rest of the treatments (Figure 8).

The total number of seeds dispersed differed significantly among treatments (F = 14.71; P = 0.000). ANE-60-LW was again the most deviant treatment: the total seed number dispersed was significantly higher than that in other treatments (Games–Howell Post-hoc test, see Figure 7). In ZOO-SMALL treatment was dispersed the lowest amount of seed, but this value was not significantly different from those in the ANE-30-WW and ZOO-BIG treatments (Figure 7).

When we considered only the wind speed and not the direction (leeward *vs.* windward), the number of dispersed seeds collected by traps could be regarded as the total number of released seeds. Following this assumption, significantly more seeds were released by wind at 60 km/h than by wind at 30 km/h (F = 23.49; P = 0.000).

DISCUSSION

Most previous studies on anemochorous species have highlighted a direct relationship between wind speed and seed abscission or release (Greene, 2005; Schippers & Jongejans, 2005; Treep et al., 2018 and references therein). In *S. canina*, it needs to be clarify. Seed abscission (i.e., separation of a seed from the mother plant) occurs without seed release (i.e., seed liberation from the fruit), because seeds stay inside the fruit. Seed release is enhanced by vibration of fruiting branches, i.e, with higher wind speed as shown for *S. canina*.

By contrast, a high RH can prevent seed abscission by hindering seed detachment from the placenta and its release (Greene, Quesada, & Calogeropoulos, 2008; Jongejans, Pedatella, Shea, Skarpaas, & Auhl, 2007). In addition, in boleochorous (considered as a type of anemochory for most authors) species, under dense fog or rain, the mature capsule can be closed. This can prevent or greatly reduce seed dispersal (Brzosko et al., 2017), even under strong wind conditions, as observed in *S. canina*. The xerochasic effect of the capsule of this species determines this behavior, and it is common in other Scrophulariaceae (Sernander, 1906). However, for *Phyteuma* species and other genera in the Campanulaceae, the size of the opening can increase with fruit age, as described by Maier, Emig, & Leins (1999). In those species, the pores of the capsules enlarge with age, allowing for greater seed shedding.

Our results showed that wind promoted and humidity hindered seed release from *S. canina*. Thus, seed release is non-random and is biased towards higher wind speeds and dry conditions (see Soons & Bullock, 2008; Greene & Quesada, 2011). This led to increased LDD, although not at the migration rates as reported by other authors (Greene, 2005; Skarpaas, Auhl, & Shea, 2006; Bohrer, Katul, Nathan, Walko, & Avissar, 2008; Soons & Bullock, 2008; Wright et al., 2008; Greene & Quesada, 2011; Nathan et al., 2011; Savage, Borger, & Renton, 2014). Instead, secondary seed dispersal was responsible for increased migration rates (author's personal observation).

Spatial primary seed dispersal

For the population of *S. canina* studied here and for the neighboring landscape the wind direction was very variable (Weather Underground, 2018). Therefore, there was not a consistent directional seed bias at population level (anisotropy) (Rodríguez-Riaño et al., 2017, 2018), unlike the situation in landscapes with relatively constant prevailing winds, such as sea breezes (Greene, Quesada, & Calogeropoulos, 2008). Simulation of prevailing winds using a blower had an anisotropic effect at the individual level, especially with extremely strong winds (60 km/h). Thus, windward seed dispersal curves were very different from leeward seed dispersal curves. The individuals of *S. canina* bearing current-year and previous-years fruiting branches (Rodríguez-Riaño et al., 2017) would reduce the wind speed, thereby decreasing the vibration of fruiting branches. In addition, the probability of seed collision with the fruiting branches would be increased. These last two effects could mask the effect of medium/high wind speeds (30 km/h), but not those of strong winds (60 km/h). These effects, whether imposed by the plant itself or by the neighboring vegetation, have been described in several studies (Bullock, Moy, Coulson, & Clarke, 2003; Bullock & Moy, 2004).

Previous studies have shown that, for *S. canina* and other species, plant structure influences the seed shadow outline (Bastida & Talavera, 2002; López-Vila & García-Fayos, 2005; Rodríguez-Riaño et al., 2017). The results in this study maintained this influence, but it usually resulted in the formation of two seed peaks, one at the beginning of the body, and one at the tail of the seed shadow. The peak in the body was greater than that in the tail, except in the 60-LW treatment where the two peaks were similar. The tail peak was mostly due to seeds from the leeward fruiting branches, and the body peak was due to seeds from the windward fruiting branches. Both showed a seed collision effect.

Natural dispersal in *S. canina* is represented by a typical leptokurtic curve (Rodríguez-Riaño et al., 2017). This pattern used to be considered as negative for plant establishment because it favors sibling competition and pathogen attack (Howe & Smallwood, 1982 and references therein; Augspurger & Kitajima, 1992; Willson & Traveset, 2000). In addition, SDD would not favor the colonization of other suitable sites because seeds are unable to cross the surrounding unfavorable sites (Quilichini & Debussche, 2000). Conversely, LDD events would overcome these physical barriers to allow the colonization of new sites, facilitate species spread, and connect fragmented populations (Tamme et al., 2014). Most of the time, LDD occurs in response to certain environmental conditions (such as updraft wind gusts and/or horizontal winds) that allow for non-random dispersal (Greene, 2005; Bohrer, Katul, Nathan, Walko, & Avissar, 2008; Soons & Bullock, 2008; Greene & Quesada, 2011; Savage, Borger, & Renton, 2014).

Many studies have shown that wind gust speed and orientation influence dispersal distances and/or the number of seeds released (Greene, 2005; Schippers & Jongejans, 2005; Skarpaas, Auhl, & Shea, 2006; Bohrer, Katul, Nathan, Walko, & Avissar, 2008; Greene, Quesada, & Calogeropoulos, 2008; Soons & Bullock, 2008; Savage, Borger, & Renton, 2014). On one hand, our results obtained using horizontal wind gusts showed that wind direction had significant effects on several seed shadow parameters, not only the distance reached by seeds. On the other hand, wind speed increased the number of seeds released. This corroborated the general rule suggested by Soons & Bullock (2008) that the higher wind speed, the more seeds will be released. This rule applies to both diaspore types (specialized and unspecialized).

Despite the increased distance of seed dispersal (up to 250 cm) with higher wind speeds, these distances are still considered as SDD. Some of the fruit features and the type of dispersion (boleochory) that could explain the shorter distribution distances with decreasing wind speed are as follows: (i) the septicidal capsule with its elongated and narrow apical opening hindered seed output; (ii) seed release required vibration of the fruiting branch, which increased with higher wind speed; (iii) seed release was not always downwind; (iv) seeds collided with current-year and older fruiting branches after release.

No previous studies have focused on dispersal of seeds from boleochorous species by animals (but see Bullock, Moy, Coulson, & Clarke, 2003). When this occurs, the species could be considered semachorous (Bonn et al., 2000). Animals are always related to endozoochory or epizoocory, that is, the dispersal of specialized diaspores, and are probably the most important vectors for LDD (Willson, 1993; Vittoz & Engler, 2007). In the boleochorous species *S. canina*, with unspecialized diaspores, the animals resulted in SDD due to rubbing or trampling the fruiting branches during their passage (see Bullock, Moy, Coulson, & Clarke, 2003 for *Rhinanthus minor*, Orobanchaceae). In addition to the wind, animals can also cause the vibration of fruiting branches; therefore, this species should be considered semachorous, rather than boleochorous, as previously classified (Rodríguez-Riaño et al., 20017).

The seed shadows produced by animal vectors differed from those produced by wind. More seeds were distributed close to the plant canopy edge than around the plant stem. The plant size and the large number of fruiting branches constituted a physical barrier to the passage of animals. Thus, animals only made contact with the peripheral fruiting branches when they rubbed against the plant, and so most of the seeds fell into the canopy-edge zone under the plant.

Seed dispersal is usually a two-step process and sometimes a multi-step process (Ozinga, Bekker, Schaminée, & van Groenendael, 2004), although more than one vector can carry out each step. Primary dispersal, which is considered to be predominant, is usually SDD and is easier to detect. Secondary dispersal is the less evident and more difficult to measure, and is usually LDD (Debussche & Lepart, 1992; Nathan & Muller-Landau, 2000; Vander Wall & Longland, 2004). In *S. canina*, both primary and secondary vectors are mainly wind, accompanied by animals and rain, respectively (Rodríguez-Riaño et al., 2017; authors' personal observation). Wind is considered as a short-distance primary vector (seeds spread to 250 cm at most). Thus, it would be associated with successional processes. Secondary vectors (although not measured) would be effective for LDD and are associated with invasion processes (see Lepart & Debussche in Debussche & Lepart, 1992; Ozinga et al., 2009).

Both primary and secondary seed dispersal could explain the structure and distribution of individuals in the *S. canina* population (see supporting information Figure S1) and other populations (authors' personal observation). This pioneer species rapidly occupies abandoned or newly created sites. At our study site, the age distribution of individuals illustrated the pattern from colonization from NE to WS (Supporting information Figure S1), starting near the holm oak line (oldest and dead individuals) and extending to the road (youngest individuals).

As Koechlin in Debussche & Lepart (1992) pointed out for *Buxus sempervirens* (Buxaceae), the distribution pattern of *S. canina* may have been affected by several factors: (a) short-distance dispersal that concentrated most seeds close to the maternal plants; (b) the line of holm oaks that prevented *S. canina* from spreading to the NE; and (c) the high mortality of progeny under the oldest individuals. Only secondary LDD could explain the subpopulation across the road. In this way, SDD generates patchy patterns that allows successional processes to avoid population extinction, while LDD allows invasion to occur (Debussche & Lepart, 1992; Kéfi, van Baalen, Rietkerk, & Loreae, 2008).

Climate change, habitat fragmentation, and other ecological factors have a great impact on species distribution. Natural seed dispersal measurement requires time-consuming quantitative fieldwork, which could be avoided by prediction of seed dispersal using mechanistic models (Soons & Bullock, 2008; Nathan et al., 2011; Tamme et al., 2014; among others). For such mechanistic models to be accurate, it is important to have a good knowledge of the intrinsic and/or extrinsic factors influencing seed dispersal. In addition, this type of work should differentiate between anemochorous species, that is, with exposed and generally specialized diaspores, and boleochorous/semachorous species, with unspecialized diaspores (generally seeds) inside a dehiscent fruit. For this reason, although it is time-consuming, fieldwork is essential to understand all the characteristics determining seed dispersal (RH, plant structure, fruiting branch density, fruit type and opening, fruit disposition in the branches, diaspore size, etc.).

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Figure legends

FIGURE 1 Seed dispersal treatments for *Scrophularia canina*. (a) Anemochorous seed dispersal. (b) Zoochorous seed dispersal simulating a big wild animal (dog about 30 kg). (c) Zoochorous seed dispersal simulating a small wild animal (dog about 3 kg). *, windward trap (WW); **, leeward trap (LW).

FIGURE 2 Total number of seed dispersed in the replicates performed using *Scrophularia canina* after different dispersal seed treatments. ANE-30-WW, windward anemochory simulating wind gusts of about 30–35 km/h. ANE-30-LW, leeward anemochory simulating wind gusts of about 30–35 km/h. ANE-60-WW, windward anemochory simulating wind gusts of about 60–70 km/h. ANE-60-LW, leeward anemochory simulating wind gusts of about 60–70 km/h. ZOO-BIG, zoochory simulating a big wild animal (dog about 30 kg); ZOO-SMALL, zoochory simulating a small wild animal (dog about 3 kg). Black line, relative humidity (RH) at 10:00 am (GMT + 1) (beginning of experiments); black dotted line, RH at 17:00 pm (GMT +1) (end of experiments); yellow line, presence of intense (solid line) or light (dotted line) fog.

FIGURE 3 Seed shadows of *Scrophularia canina* after different dispersal seed treatments. (a) Natural seed dispersal (data from Rodríguez-Riaño et al., 2017). (b) Windward anemochory simulating wind gusts of about 30–35 km/h. (c) Leeward anemochory simulating wind gusts of about 30–35 km/h. (d) Windward anemochory simulating wind gusts of about 60–70 km/h. (e) Leeward anemochory simulating wind gusts of about 60–70 km/h. (f) Zoochory simulating a big wild animal (dog about 30 kg). (g) Zoochory simulating a small wild animal (dog about 3 kg). Treatment abbreviations and line colors as in Figure 2. Vertical lines show average plant lateral spread.

FIGURE 4 Seed distribution patterns (ln-transformed) of *Scrophularia canina* after different dispersal seed treatments and natural dispersal fitted to a linear function. Insets down left of each figure show fit to quadratic function (on left for natural dispersal). (a) Natural seed dispersal (data from Rodríguez-Riaño et al., 2017). (b) Windward anemochory simulating wind gusts of about 30–35 km/h. (c) Leeward anemochory simulating wind gusts of about 30–35 km/h. (d) Windward anemochory simulating wind gusts of about 60–70 km/h. (e) Leeward anemochory simulating wind gusts of about 60–70 km/h. (f) Zoochory simulating a big wild animal (dog about 30 kg). (g) Zoochory simulating a small wild animal (dog about 3 kg). Treatment abbreviations and line colors as in Figure 2.

FIGURE 5 Schematic representation of seed shadow parameters of different dispersal treatments and natural dispersal in *Scrophularia canina*. (a) Modal distance, seed percentage underneath plant's lateral spread, and maximum distance reached by a seed. (b) Dispersal distance percentiles. Black dashed line in (b) indicates plant lateral spread. Natural (natural seed dispersal data from Rodríguez-Riaño et al., 2017). Treatment abbreviations as in Figure 2.

FIGURE 6 Box-plot of different seed shadow parameters from six dispersal treatments and natural seed dispersal in *Scrophularia canina*. (a) 25th percentile. (b) 50th percentile. (c) 75th percentile. (d) 90th percentile. (e) 99th percentile. (f) Max. distance (maximum dispersal distance). Different letters above boxes indicate significant differences ($P < 0.05$). 30-WW, windward anemochory simulating wind gusts of about 30–35 km/h; 30-LW, leeward anemochory simulating wind gusts of about 30–35 km/h; 60-WW, windward anemochory simulating wind gusts of about 60–70 km/h; 60-LW, leeward anemochory simulating wind gusts of about 60–70 km/h; BIG, zoochory simulating large wild animal (dog about 30 kg); SMALL, zoochory simulating small wild animal (dog about 3 kg); NAT (natural seed dispersal data from Rodríguez-Riaño et al., 2017). Colors as in Figure 2.

FIGURE 7 Box-plot of different seed shadow parameters from six dispersal treatments and natural seed dispersal in *Scrophularia canina*. (a) % seed (seed percentage under plant canopy). (b) Skewness index. (c) Slope (slope of linear regression of seed number; ln-transformed). (d) Kurtosis index. (e) Modal distance (distance reached by greatest number of dispersed seeds). (f) Total seed number (total number of seeds collected by traps in all replicates of each treatment per individual). Different letters above boxes indicate significant differences ($P < 0.05$). NAT (natural seed dispersal, data from Rodríguez-Riaño et al., 2017, 2018). Treatment abbreviations and colors as in Figure 6.

FIGURE 8 Dendrograms obtained from clustering analysis by Ward's method based on Euclidean distance squared. Dendrograms group six different seed dispersal treatments and natural seed dispersal in *Scrophularia canina*. (a) Dendrogram based on all studied parameters (25th, 50th, 75th, 90th, and 99th percentiles, seed percentage under plant canopy, modal distance, maximum dispersal distance, slope, and skewness and kurtosis indices). (b) Dendrogram based on all parameters except for maximum dispersal distance. (c) Dendrogram based on all parameters except for 50th percentile. Natural (natural seed dispersal, data from Rodríguez-Riaño et al., 2017, 2018). Treatment abbreviations as in Figure 2.

Supporting information

FIGURE S1 Spatial distribution of different types of individuals according to their reproductive maturity state and vegetative development in studied population of *Scrophularia canina* (Source: Google Earth). White line, expansion zone constituted by relatively isolated fertile young and old mature individuals; blue line, clumps of old fertile individuals; red line, clumps of dead individuals; orange line, holm oak individuals adjacent to small watercourse; yellow line, clumps of retama and/or rockrose; green line, holm oak with rockrose vegetation.

TABLE S1 Spearman correlation coefficients between studied seed shadow parameters in different dispersal treatments and two plant traits.

Effects of different abiotic and biotic factors on spatial primary seed dispersal in the semachorous species *Scrophularia canina*

Plant Species Biology

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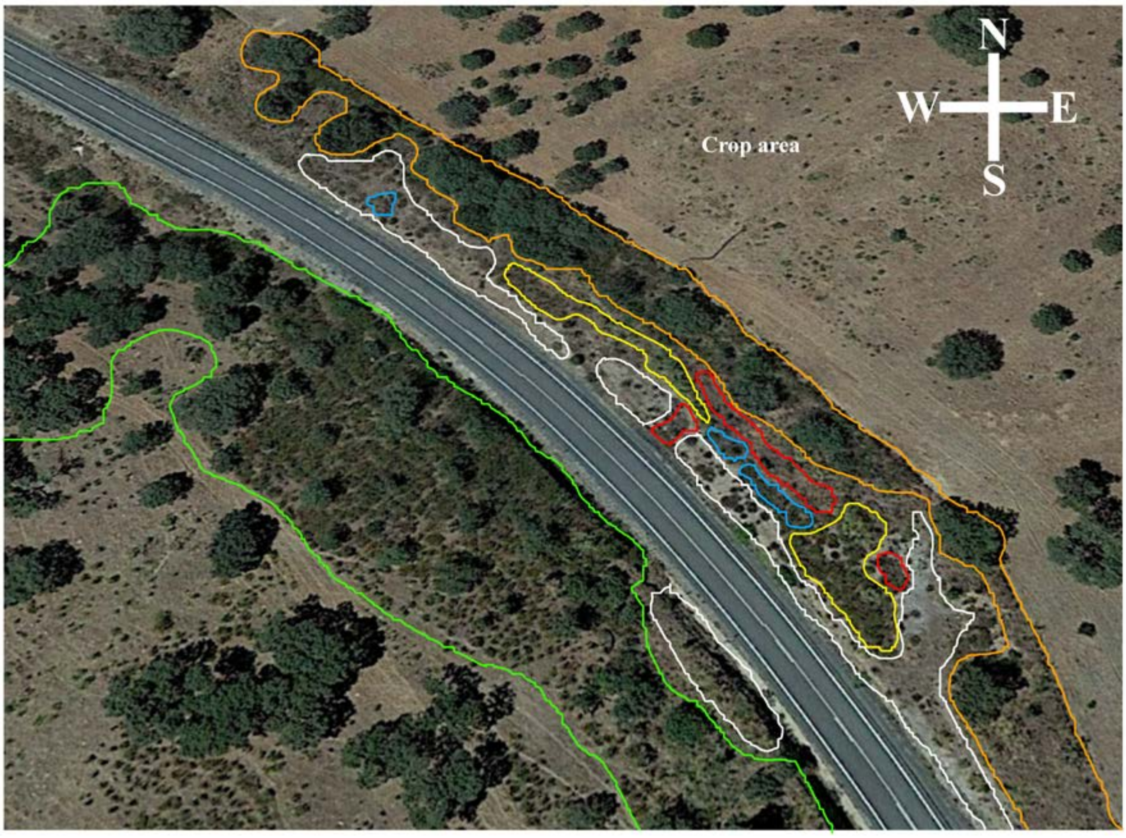

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Table S1. Spearman correlation coefficients between studied seed shadow parameters in different dispersal treatments and two plant traits.

	Anemochory								Zoochory				Natural	
	30 km/h				60 km/h				BIG		SMALL			
	Windward		Leeward		Windward		Leeward							
	Height	Lateral spread	Height	Lateral spread	Height	Lateral spread	Height	Lateral spread	Height	Lateral spread	Height	Lateral spread	Height	Lateral spread
25th	0.24^{ns}	0.33^{ns}	0.43^{ns}	-0.01^{ns}	0.33^{ns}	**0.54***	**0.53***	0.20^{ns}	-0.01^{ns}	0.21^{ns}	0.12^{ns}	0.36^{ns}	−0.138^{ns}	**0.506*****
50th	0.31^{ns}	0.36^{ns}	0.46^{ns}	-0.08^{ns}	0.34^{ns}	**0.66****	**0.75****	0.33^{ns}	-0.04^{ns}	0.28^{ns}	0.18^{ns}	**0.65****	−0.119^{ns}	**0.479*****
75th	0.27^{ns}	0.03^{ns}	0.38^{ns}	-0.04^{ns}	0.37^{ns}	**0.54***	**0.80*****	0.44^{ns}	-0.30^{ns}	-0.02^{ns}	0.40^{ns}	**0.66****	−0.094^{ns}	**0.547*****
90th	0.40^{ns}	0.02^{ns}	**0.52***	0.11^{ns}	0.25^{ns}	0.13^{ns}	**0.75****	0.40^{ns}	-0.23^{ns}	-0.12^{ns}	0.30^{ns}	**0.81*****	−0.094^{ns}	0.186^{ns}
99th	**0.55***	0.19^{ns}	0.33^{ns}	0.07^{ns}	0.11^{ns}	0.04^{ns}	**0.67****	0.11^{ns}	-0.47^{ns}	-0.08^{ns}	-0.08^{ns}	0.17^{ns}	**−0.249***	0.061^{ns}
Modal	-0.17^{ns}	-0.06^{ns}	0.34^{ns}	-0.18^{ns}	-0.18^{ns}	0.27^{ns}	0.35^{ns}	-0.01^{ns}	-0.18^{ns}	0.03^{ns}	0.49^{ns}	0.47^{ns}	−0.162^{ns}	**0.225***
% Seed	0.14^{ns}	**0.74****	0.08^{ns}	**0.65****	0.14^{ns}	**0.62***	-0.23^{ns}	**0.55***	0.25^{ns}	**0.77*****	0.19^{ns}	0.40^{ns}	0.158^{ns}	**0.559*****
Maximum	**0.57***	0.30^{ns}	**0.39***	0.14^{ns}	0.32^{ns}	0.24^{ns}	0.48^{ns}	0.18^{ns}	-0.29^{ns}	-0.02^{ns}	-0.09^{ns}	0.25^{ns}	-0.40^{ns}	0.01^{ns}
Skewness	0.41^{ns}	-0.03^{ns}	-0.33^{ns}	0.22^{ns}	-0.35^{ns}	**0.58***	**-0.78*****	-0.34^{ns}	0.25^{ns}	0.04^{ns}	-0.31^{ns}	**-0.79*****	−0.150^{ns}	**0.306****
Kurtosis	0.37^{ns}	0.15^{ns}	**-0.67****	-0.04^{ns}	0.35^{ns}	**-0.58***	-0.05^{ns}	0.00^{ns}	0.30^{ns}	0.04^{ns}	-0.26^{ns}	**-0.66****	**−0.249***	0.167^{ns}
Slope	0.08^{ns}	-0.01^{ns}	0.20^{ns}	-0.33^{ns}	0.24^{ns}	-0.02^{ns}	0.42^{ns}	0.11^{ns}	-0.15^{ns}	0.07^{ns}	0.22^{ns}	0.46^{ns}	−0.207^{ns}	0.104^{ns}

Natural dispersal data from Rodríguez-Riaño et al. (2017, 2018).

25th, 50th, 75th, 90th, and 99th percentiles correspond to distance reached by 25%, 50%, 75%, 90%, and 99% of dispersed seeds, respectively.

% Seed: percentage of seeds beneath each plant's lateral spread.

Maximum: maximum distance reached by a seed.

Modal: modal dispersal distance (distance reached by greatest number of dispersed seeds).

Slope: slope of linear regression of seed number (ln-transformed).

***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant.