Temporal and spatial intraspecific variation of primary seed dispersal in *Scrophularia canina* L., a widespread plant with unspecialised diaspores

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Background: As seed dispersal can vary among years and individuals, studies that focus on a single year or on a few individuals may lead to erroneous conclusions.

Aims: To study temporal and spatial intraspecific variation of seed dispersal in *Scrophularia canina*, a widespread species with capsule-type fruit.

Methods: Primary seed dispersal was quantified by placing traps in each cardinal direction around 10 individuals during two consecutive years. We correlated several seed shadow parameters (modal dispersal distance, kurtosis, skewness, percentiles, slope, and seed percentage beneath the plant canopy) with three plant features (maximum height, lateral spread and seed production).

Results: Scrophularia canina dispersed their seeds by boleochory, giving rise to a typical leptokurtic curve, but behaving as a barochorous species, because about 90% of seeds landed beneath the plant canopy. Temporal dispersal in *S. canina* included several seed waves associated with maximum wind speeds. Plant lateral spread was significantly positively correlated with seed percentiles and percentage of seeds beneath the plant canopy regardless of year. A seed production effect was only evident when both years were considered together.

Conclusions: Although time-consuming, investigation of the dispersal process for more than one year provides more realistic information on seed dispersal. Lateral spread is the main plant feature determining seed shadow.

Keywords: Boleochory; mother plant lateral spread; percentiles; *Scrophularia canina*; spatial seed dispersal; temporal seed dispersal

Introduction

Diaspore dispersal is a crucial step in the plant life cycle. Dispersion patterns determine the distribution of individuals in a population, the probability of incorporation of new individuals and the potential for establishment of new populations in suitable habitats (Howe and Smallwood 1982, and references therein; Willson and Traveset 2000; Wenny 2001; Wang and Smith 2002). Diaspore dispersal can enhance plant fitness by reducing the density-dependent mortality of seeds or seedlings (e.g., sibling competition, predation and pathogen attacks) near the maternal plant (Janzen 1970; Howe and Smallwood 1982, and references therein; Augspurger 1983a; Augspurger and Kitajima 1992; Willson and Traveset 2000) and is a key process related to species evolution (Clobert et al. 2012).

The primary dispersal process, more precisely, dispersal distance and seed shadow, is influenced by diaspore type as well as maternal plant traits connected to different vectors and environmental variables (e.g., Tackenberg et al. 2003; Muller-Landau et al. 2008; Thomson et al. 2011).

As diaspore dispersal is undoubtedly advantageous, plants try to maximise this process. In theory, such an enhancement can be achieved in various ways, such as by improving diaspore adaptations to dispersal (e.g., Willson 1993; Willson and Traveset 2000; Wenny 2001; Vittoz and Engler 2007; Thompson et al. 2011) and/or by combining two or more dispersal mechanisms (Kaufmann et al. 1991; Griffith and Forseth 2002; Andresen and Feer 2005; López-Villa and García-Fayos 2005; Castro et al. 2010).

Except in the case of autochorous plants, diaspore adaptations function to facilitate transport by external dispersal agents such as wind, animals or water (Jongejans and Telenius 2001; Vittoz and Engler 2007, and references therein). Even in no autochorous species, however, diaspores lacking accessories to facilitate transport—at least in communities of temperate climates —are as common as those with adaptations (Willson et al. 1990). Nevertheless, most dispersal studies have focused on plants with diaspores having accessories (review in Willson 1993; Donohue 1998; Quilichini and Debussche 2000; López-Vila and García-Fayos 2005; review in Vittoz and Engler 2007; Castro et al. 2010; Skarpaas et al. 2011), with a focus on specialised anemochorous plants, which typically show a leptokurtic distribution around the mother plant after primary seed dispersal (Howe and Smallwood 1982; Willson and Traveset 2000). Furthermore, most

studies have used standardised experiments to create mathematical simulations for predicting dispersal curves, thereby avoiding time-consuming quantitative field work (Augspurger and Franson 1987; Matlack 1987; Greene and Johnson 1996; Strykstra et al. 1998; Maier et al. 1999; Tackenberg 2003; Schurr et al. 2005; Tamme et al. 2014; Trakhtenbrot et al. 2014). Although indeed time-consuming, field work is necessary because it encompasses plant features (plant height, lateral spread, fruiting branch arrangement, fruit type and seed size), population or microhabitat characteristics, environmental variables (wind direction, velocity and rainfall pattern) and the behaviour of dispersing animals—all factors that can affect diaspore dispersal. In other words, field studies are required to add realism to modelling results (e.g., Thiede and Augspurger 1996; Quilichini and Debussche 2000; Griffith and Forseth 2002; López-Vila and García Fayos 2005 Thomson et al. 2010; Tamme et al. 2014).

Most studies have analysed spatial variation in seed dispersal (seed shadow) and compared different species within genera (Maier et al. 1999; Jongejans and Telenius 2001) or between genera (Debussche and Lepart 1992; Willson 1993; Vittoz and Engler 2007; Thomson et al. 2011). By contrast, temporal variation (Nathan and Muller-Landau 2000; Robledo-Arnuncio et al. 2014) and intraspecific spatial variation (McCanny and Cavers 1987, 1989; Augspurger 1983a, b; Hoppes 1988; Thiede and Augspurger 1996) have less frequently been examined. Although limited, such studies have demonstrated that maternal architecture can affect dispersal variation among individuals (McCanny and Caves 1989; Thiede and Augspurger 1996).

In this context, our goal was to study the dispersal of *Scrophularia canina*, a widespread species with a capsule-type fruit that produces seeds that lack conspicuous adaptations for dispersal. We aimed to quantify spatial and temporal seed dispersal, determine the extent of intraspecific variability among maternal plants and between years, and clarify whether the architecture of individual plants contributes to variation in seed dispersal.

Material and methods

Plant species and study area

Scrophularia canina (Scrophulariaceae) is a suffruticose species that flowers from March to June. It inhabits subnitrophilous communities from southern and central Europe, southern Russia, northern Africa, Anatolia, north-western Iran and south Asia

to the western Himalayas, and is naturalised in Britain (Ortega-Olivencia 2009) and the USA (USDA 2016). The flowers, which are hermaphroditic, protogynous, zygomorphic and nectariferous, are arranged in dichasial cymes constituting a paniculate inflorescence. The corolla is bilabiate, small (3–5.5 mm) and violet. Four stamens adnate to the base of the corolla lower lip are present, with a linear or narrowly linear-lanceolate whitish or purple staminode (0.4–1 to 1.6) mm), sometimes absent, inserted in the lower third of the corolla upper lip. The main pollinators are hoverflies (Valtueña et al. 2013; Rodríguez-Riaño et al. 2014). The fruits are ovoid-suborbicular, persistent capsules (3–7 mm), slightly acuminate and harbouring blackish seeds (0.6–1.7 mm) (Ortega-Olivencia 2009).

The studied population was in south-western Europe (Badajoz Province, Extremadura, Spain). The experimental site was situated along the road from Alburquerque to Villar del Rey (39° 11′ 6.20″ N–6° 56′ 14.83″ W) between 313 and 320 m a.s.l. (Figure S1). The vegetation between the road and the adjoining stream bank was a scrub dominated by *S. canina* and scattered individuals of *Quercus rotundifolia* and *Retama sphaerocarpa* (López et al. 2016). The average annual temperature was 15.8 °C and the annual precipitation was 652 mm (Weather Underground 2016). The study was carried out on 10 selected individuals during two consecutive years: from October 2013 to August 2014 (first year) and from September 2014 to June 2015 (second year). The selected individuals were sufficiently isolated from neighbouring plants to ensure identification of diaspore origin (Figure S1).

Individual characteristics

To study abiotic primary dispersal (see below) in each of the selected individuals, we (1) measured maximum height at the centre of each plant and lateral spread (i.e., the zone beneath the branches of the individuals) for each principal compass direction and (2) estimated seed production per individual. For this estimation, we counted the number of fruiting branches from the current year. Fruiting branches from several previous years also remained on individual plants. We recorded the number of capsules on four of these branches. The product of the mean capsule number and the number of fruiting branches corresponded to an estimate of the number of capsules per individual. Finally, we multiplied the latter number by the mean seed number per capsule (9.7

seeds/capsule; Ortega-Olivencia and Devesa 1993). All plant traits, except for plant lateral spread, were analysed by a one-way repeated-measures ANOVA with year as within-subject variable. Plant lateral spread was analysed by two-way repeated-measures ANOVA with year and compass direction as within-subject variables. In both cases the sphericity hypothesis was assumed. All statistical analyses were carried out using the SPSS statistical package (IBM Corp. 2010).

Abiotic primary dispersal

We painted a compass rose on the ground with white paint around each individual to mark the principal cardinal points (Figure 1a). To evaluate seed fall, we nailed 100-cm long sticky seed traps to the ground in each principal cardinal direction, with one end of the trap situated at the base of the plant and labelled as 0 cm (Figure 1b). Each trap consisted of 5-cm-wide tape that was stapled sticky side up to a 100×10 cm fibreboard, painted white, to facilitate observing the blackish seeds (Figure 1c). Trap efficacy was verified before use to ensure that seeds firmly bonded to the tape. In addition, we checked for maximum dispersal distance using 150-cm long traps. As dispersal distances never exceeded 100 cm, we trimmed the seed traps to this length.

After placement, traps were left for 5–6 days and then collected, labelled and transported to the laboratory. For transportation to the laboratory, 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 millimetre paper, seed number per centimetre was counted. Once counted the total seed number in each sample was divided by the number of days to derive the mean seed number per sample per day.

Seed shedding was measured 25 times: 12 times during the first year and 13 during the second one.

Temporal primary dispersal

Temporal distribution of seed dispersal was analysed by using a repeated-measures ANOVA, with individual as the principal factor and compass direction as the covariate. Because the sphericity hypothesis had to be rejected, we used the univariate *F* statistic

and applied a Huynh-Feldt epsilon correction (Huynh and Feldt 1976). Maximum wind speed on days of trap placement was obtained from the meteorological station of Talavera la Real (Badajoz), which was located nearly 37 km southeast of the study area.

Spatial primary seed dispersal

Seed shadow distribution for each individual plant and compass direction was represented and analysed by Generalised Estimating Equations (GEE) fitted to a gamma distribution (i.e., dispersal curves strongly skewed to the left) with a logarithmic link function, using year, compass direction and distance to the plant stem as within-subject variables.

To index dispersal capacity and seed distribution patterns (seed shadow), we used the three parameters mentioned by Willson (1993), namely, (1) slope of the linear regression of seed number (ln-transformed), (2) modal dispersal distance—the distance reached by the greatest number of dispersed seeds and (3) maximum dispersal distance—the distance reached by 99% of dispersed seeds. To these parameters we added 25th, 50th, 75th and 90th percentiles of dispersal distance, the percentage of seeds underneath the plant's lateral spread, the maximum distance reached by a seed and kurtosis and skewness indices.

Differences among individuals of the studied seed shadow parameters were analysed as follows. Percentiles, and modal dispersal distance were analysed using GEE fitted to a binomial negative distribution with a logarithmic link function. However, slope, kurtosis, skewness and the percentage of seeds underneath the plant's lateral spread were compared by two-way repeated-measures ANOVA. Year and compass direction were treated as within-subject variables. Kurtosis was normalised by log (kurtosis+1)-transformation.

The above-mentioned seed dispersal parameters (slope of the linear regression, percentiles, modal dispersal distance and skewness and kurtosis indices) were correlated with plant traits (maximum height, lateral spread and seed production) using the Spearman linear correlation. To test these correlations, we considered each sample collected at a principal compass direction to be independent of other samples from the same individual. We did this on the basis of the following: (1) most studied seed shadow parameters calculated from sample data for a given individual and year were

not correlated among compass directions, with absolutely no correlation occurring in both years (not shown) and (2) the architecture of the mother plant resulted in different fruiting branches oriented towards each compass direction (see Figure 1). In addition, we considered seed production per individual to be homogeneously distributed among the different compass directions.

Results

Seed dispersal in *S. canina* is by boleochory or semachory, i.e., seeds are dispersed by the action of any vector (wind, animals or rain) that can violently shake the rigid branches sustaining the capsules. All studied plant features related to fertility were significantly higher in the first year (Table 1). Considering plant architectural traits, the individuals were significantly taller in the first year and more laterally spread in the second (Table 1).

Abiotic primary dispersal

Temporal primary dispersal. Temporal seed dispersal in *S. canina* is an uninterrupted process between consecutive years (Figure 2). There is a weak overlap in September, in which the plants could disperse the few remaining seeds in the last year's capsules and those seeds from the new capsules of the current year.

As shown in Figure 2a, the overall dispersal curve was characterised by several peaks and troughs in each year. The height and timing of the peaks varied by year. Two large peaks occurred during the first year—one at the beginning of the dispersal process and another smaller one in April—in addition to two barely differentiated peaks (Figure 2a). Four peaks were also evident during the second year. In contrast to the first year, all four of these peaks were very similar to one another and were less pronounced than the two large peaks in the first year; the largest occurred in mid-January (Figure 2a). A relationship between seed dispersal and maximum wind speed was also apparent (Figure 2a). In most cases, maximum wind speed peaks preceded or were simultaneous with seed dispersal peaks (note distance from the weather station to the study site).

The dispersal curves were practically identical with respect to cardinal direction (Figure 2b). Regardless of whether or not compass direction was taken into account

(Figures S2 and 2c, respectively), the temporal dispersal curves seemed very different at the individual plant level; thus, the mean number of dispersed seed in the dispersal peaks ranged from 3.75 to 33.45 in the first year and from 0.25 to 16.86 in the second (for more details see Figure 2c). In most individuals, however, the timing of the different dispersal peaks roughly coincided.

As expected, temporal primary dispersal (i.e. dispersed seed number) significantly depended on census date and its interaction with the individual (Table 2). However, the census-compass direction interaction was only significant on the second year (Table 2) in which individuals were significantly spreader laterally (Table 1).

Spatial primary dispersal. The overall spatial pattern of primary seed dispersal followed a typical leptokurtic distribution (Figure 3), with most seeds dispersed close to the main stem of the plant (thereafter, plant stem). First-year curves (Figure 3a) were more strongly leptokurtic than those from the second year (Figure 3b). Year, compass direction and distance to the plant stem significantly influenced this spatial dispersion pattern (Table 3). No seed was dispersed more than 100 cm from the plant.

Skewness and kurtosis. The lowest and highest values were obtained during the second year. Except for three negative kurtosis values, both kurtosis and skewness were always positive (Table S1).

Skewness and kurtosis were not significantly affected by either year or compass direction (Table 4). Lateral spread was the only plant trait significantly negatively correlated with both indices, but lacking signification during the first year (Table 5).

Slope. Slopes for the first year were always steeper than those for the second year (Table S1 and Figure 4), considering both the compass direction with individual factor removed (Table S1 and Figure S3) as at the individual level (Figure S4).

The slope related to seed shadow was significantly affected by year, but not by compass direction (Table 4). In addition, slope was negatively correlated with seed production, but only significantly, when both years were considered together (Table 5).

Modal dispersal distance. In most cases, the modal dispersal distance was closer to the plant stem in the first year than in the second. During the first year, this distance was 3 cm or less in 82.5% of cases and never exceeded 10 cm. During the second year, 60% of modal dispersal distances were 3 cm or less, with a maximum observed value of 38 cm.

Modal dispersal distance was significantly influenced by year and its interaction with compass direction (Table 4). In addition, this parameter was significantly positively correlated with plant lateral spread and negatively with seed production, i.e., the modal dispersal distance was shortest when seed production was highest (Table 5), but only when both years were considered together.

Percentiles. In both years and even considering both together, 25th, 50th and 75th percentiles of dispersal distance fell under individual plant canopy, with the 90th percentile more or less matching plant canopy and the 99th percentile clearly falling beyond it (Figure 5). These results are similar at the individual level when considering compass direction, although with some exceptions (Figure S5).

Percentiles in the second year were generally located further away from the plant stem than in the first year. In the first year, for example, the 25th percentile was reached more than 10 cm from the plant stem in only 5% of cases vs. 25% of cases in the second year (see Figure S5).

The nearest percentiles to the plant stem were significantly affected by year, while compass direction influenced on 50th, 75th and 99th percentiles (Table 4). Plant lateral spread was the only plant trait positively and significantly correlated with 25th, 50th and 75th percentiles but not correlated with 90th and 99th percentiles during one or both years (Table 5). Maximum plant height was significantly positively correlated with the 50th and 75th percentiles in the first year, and negatively with the 99th percentile when considering both years together (Table 5). Regarding seed production, the correlation was always negative although only significant for those percentiles closest to the plant stem (25th and 50th) as well as the farthest (99th), (Table 5) when considering both years together.

Percentage of seeds underneath plant lateral spreads. During both years, most seeds landed beneath canopy of each plant. In the first year, the mean proportion of seeds beneath plant canopy was 91.2% (58.2–99.2%); during the second year, the proportion was 86.8% (67.7–99.5%). This parameter was affected by year-compass direction interaction (Table 4) and it was positively significantly correlated with plant lateral spread, as for 25th, 50th and 75th percentiles (Table 5), and nearly matched the 90th percentile.

Discussion

Dispersal mode

Scrophularia canina, which undergoes primary seed dispersal by boleochory or semachory, exhibits the typical features of species with these dispersal mechanisms (Vittoz and Engler 2007). It possesses rigid fruiting branches that bear capsules with small seeds which lack dispersal devices. The only feature that would facilitate wind dispersal of the seeds is their small size. Along with this mode of abiotic primary dispersal, secondary abiotic dispersal of fallen seeds on the ground occurs via wind and/or rain (authors' personal observation); however, because of the small size of the seeds, this dispersal was impossible to measure. Although both primary and secondary seed removal by ants takes place in addition to abiotic dispersal, this ant-mediated seed removal is technically considered to be seed predation (Rodríguez-Riaño et al., unpublished data).

Seed release mechanisms have a major influence on the duration of seed dispersal (Bastida and Talavera 2002). Similar to *Cistus* species, *S. canina* has a very extended seed release period—nearly a full year (from early September to late August), with the end of the dispersal process of one year overlapping with the beginning of the next year. This characteristic is caused by the presence of septicide capsules with apical openings through which seeds, sequentially liberated from the placenta, are gradually released; these liberated seeds are only released when fruiting branches are strongly shaken, which leads to successive seed dispersal waves associated with high wind speeds (see Greene et al. 2008). This extended dispersal pattern can be interpreted as a strategy for spreading germination risk and maximising seedling establishment (Janzen 1969; Bastida and Talavera 2002).

Abiotic primary dispersal characteristics

Scrophularia canina exhibited very different temporal and spatial seed dispersal patterns between the two years studied, i.e., not all the seed shadow parameters studied had a similar pattern for each year, confirming the necessity of multiple-year studies (e.g. Nathan and Muller-Landau 2000; Robledo-Arnuncio et al. 2014).

Regarding temporal seed dispersal, the seed number dispersed was related to seed production per individual and to maximum wind speed. Thus, differences among

censuses were due to weather conditions and differences in seed production among individuals; by contrast, the absence of differences among compass directions seems to be related to a high seed production that could mask the effect of wind.

With respect to intraspecific variation in seed shadow, Thiede and Augspurger (1996) have reported that the seed shadow of *Lepidium campestre* ranges from platykurtic to leptokurtic and from non-skewed to right-skewed. In *S. canina*, by contrast, the assumption of Levin and Kerster (1974) that seed shadow distributions are right-skewed and strongly leptokurtic could be supported by our results. Most seed shadows of *S. canina* individuals were leptokurtic, as only three out of 80 samples were platykurtic. In addition, the distributions were always more or less strongly right-skewed.

All studied seed shadow parameters were higher or stronger for the first year, indicating that a greater seed crop implies greater number of seeds deposited close to the plant stem. From an ecological point of view, this pattern of dispersal enhances the density-dependent mortality near the maternal plant (Janzen 1970; Howe and Smallwood 1982, and references therein; Augspurger 1983a; Augspurger and Kitajima 1992; Willson and Traveset 2000). However, as seed dispersal in *S. canina* is a long-drawn out process and because shed seeds can be redistributed by wind or rain high seed density on the ground can be reduced.

Several characteristics of the mother plants may be related to seed dispersal. One such characteristic is plant architecture (McCanny and Cavers 1989; Bastida and Talavera 2002; López-Vila and García-Fayos 2005). As mentioned above, seeds in boleochorous species such as *S. canina* are dispersed when fruiting branches are shaken by wind or other vectors (Vittoz and Engler 2007). In their calculations, Soons and Ozinga (2005) obtained very short dispersal distances (< 0.5 m), but did not consider a catapult effect. By considering a catapult effect and stem size, Vittoz and Engler (2007) calculated distances ranging from almost 1 m for low-stature species (< 30 cm tall) to 3–5 m for tall species (> 30 cm tall). All the species studied by Vittoz and Engler (2007) are architecturally very different from *S. canina*. Most are herbaceous with few fruiting branches, or, less frequently, shrubs with fruits located at the branch tips so that seed release is not hampered by neighbouring branches. By contrast, *S. canina* is a sub-shrubby species of about 90 cm high; its dispersal distances are no more than 100 cm from the centre of the mother plant, an even lower range than that given for small

species by Vittoz and Engler (2007). This limited range may be due to the architectural peculiarity of *S. canina* individuals. In these individuals, dry fruiting branches retained from the past several years may interfere with the release of seeds from swaying fruiting branches of the current year; in other words, released seeds may crash into old or current branches and thus be slowed or halted, similar to the observations of López-Vila and García-Fayos (2005) for the dispersal of seeds from the innermost fruits of *Ulex parviflorus*. Thus, the seeds are dispersed at very short distances.

Another factor possibly contributing to these extremely short distances is fruit dehiscence type, which hinders the release of seeds at a speed sufficient to reach greater distances. If *S. canina* plants are subjected to wind speeds (60–70 km h^{-1}) higher than the maximum prevailing wind speed in the study area (ca. 30 km h^{-1}), these dispersal distances increase only up to 110 cm (Rodríguez-Riaño et al., unpublished data). Consequently, both individual architecture and fruit dehiscence type seem to affect these seed dispersal distances.

With respect to seed dispersal distances and seed shadow parameters, *S. canina* behaves as a barochorous species, such as species of *Cistus* (Bastida and Talavera 2002), and not as a boleochorous species (see Vittoz and Engler 2007). In barochorous plants, most seeds land beneath the plant canopy (ca. 80% in *Cistus*), with the remaining falling in the immediate vicinity of the mother plant (Heinken 2004; Bastida and Talavera 2002). In *S. canina*, up to approximately 90% of seeds landed beneath the mother plant canopy, and the rest did not exceed 100 cm.

Another architectural feature associated with seed shadow is the position of fruits in the mother plant. For example, fruits in *Cistus ladanifer* are mainly present in the outer half and upper part of the plant canopy, with the highest seed densities consequently found between 20 and 60 cm from the centre of the mother plant (Bastida and Talavera 2002). In *S. canina*, fruit position is also responsible for the seed shadow distribution. Fruits are located throughout the fruiting branches, which extend in all directions from the centre of the individual. When both years were considered together, 71.3% of samples originating within 3 cm of the mother plant centre were the modal distance, and the 25th percentile of 85% of the samples reaching up to 10 cm.

Plant height (Thiede and Augspurger 1996) and lateral spread (McCanny and Cavers 1989; Thiede and Augspurger 1996) are other plant features related to seed shadow distribution. In *S. canina*, only lateral spread was clearly correlated with some seed

shadow parameters; it was always significantly positively correlated with all percentiles, except the 90th and 99th, and percentage of seeds beneath the plant canopy regardless of year. Even when we considered both years together, the response was identical. Plant lateral spread can therefore be used to infer seed shadow in *S. canina* by estimating the 25th, 50th and 75th percentiles and the seed percentage beneath the plant canopy. Conversely, mother plant height does not seem to be a good plant trait to estimate any seed shadow parameter, despite the differences between years.

The type of primary dispersal addressed in this study, i.e., short-distance dispersal, is related to local population recruitment. By contrast, long-distance dispersal, mainly by wind (authors' personal observation), should be an important factor in either the broad distribution (see Calviño-Cancela et al. 2006) achieved by this species or its survival in fragmented landscapes (Primack and Miao 1992).

Conclusions

The seed shadow of *S. canina*, a widespread species with unspecialised diaspores, is shaped by several characteristics of the mother plant. First, the most influential characteristic is plant lateral spread, but only for seed shadow parameters located beneath the plant canopy (25th to 75th percentiles, and percentage of seeds) regardless of year. Second, individual architecture (fruiting branch traits and retention of dry branches from previous years) acts as an obstacle that hinders the effectiveness of branches being swayed by wind or another vector. Because seeds are consequently blocked by old and current fruiting branches, seed dispersal distances are shorter than those observed in other boleochorous species. Third, seed production when studied over more than one year can provide insight into the seed shadow. Conversely, mother plant height, because of to be year dependent, is not a good feature for inferring the seed shadow. In conclusion, the study of dispersal processes over more than one year, even though time-consuming, provides a more realistic scenario of the correlation of certain plant architectural features with seed shadow.

Disclosure statement

No potential conflict of interest was reported by the authors.

Supplemental data

Supplemental data for this article can be accessed here.

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The research team studies animal pollination, role of the staminode, dispersal, systematics, evolution and phylogeography in western Mediterranean and Macaronesian plants, focused in the last times in different species of the genus *Scrophularia.*

Author contributions: T.R.R. and A.O.O. had the original ideas and designed experiments. T.R.R., J.L.P.B., J.L., F.J.V. and A.O.O. carried out field experiments. T.R.R. and M.G. performed statistical analyses. T.R.R. wrote the manuscript with contributions from A.O.O. All enhanced and edited the manuscript.

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Figure legends

Figure 1. Seed trapping in *Scrophularia canina,* Badajoz Province, Extremadura, Spain, 2013-2015. (a) Single individual of *S. canina* surrounded by painted principal and intermediate compass directions. The blue line bisecting each paint strip indicates the lateral spread of the plant in that compass direction. (b) Single individual with traps placed in the principal compass directions. (c) Detail of a trap 5 days after placement under an individual showing seeds glued to the sticky tape.

Figure 2. Temporal pattern of seed dispersal in *Scrophularia canina*, Badajoz Province, Extremadura, Spain, 2013-2015. (a) Seed number per sample collected in traps (mean + SD). (b) Mean seed number per sample and per principal compass direction collected in traps. (c) Mean seed number per sample and per individual collected in traps; the range for the main dispersal peaks is shown.

Figure 3. Seed shadow in *Scrophularia canina*, Badajoz Province, Extremadura, Spain, 2013-2015. (a–c) Mean seed number collected in traps by principal compass direction during first year (a), second year (b) and both studied years (c). (d) Mean seed number collected in traps per year. Vertical lines show the average plant lateral spread.

Figure 4. Seed distribution pattern (ln-transformed) in *Scrophularia canina*, Badajoz Province, Extremadura, Spain, 2013-2015. Seed distribution during first year (a), second year (b) and both year combined (c).

Figure 5. Schematic representation of dispersal distance percentiles by compass direction in *Scrophularia canina*, Badajoz Province, Extremadura, Spain, 2013-2015. (a) First year. (b) Second year. (c) Both years–combined. 25th, 50th, 75th, 90th and 99th percentiles correspond to the distance reached by 25%, 50%, 75%, 90% and 99% of dispersed seeds, respectively. Black dotted lines indicate plant lateral spread.
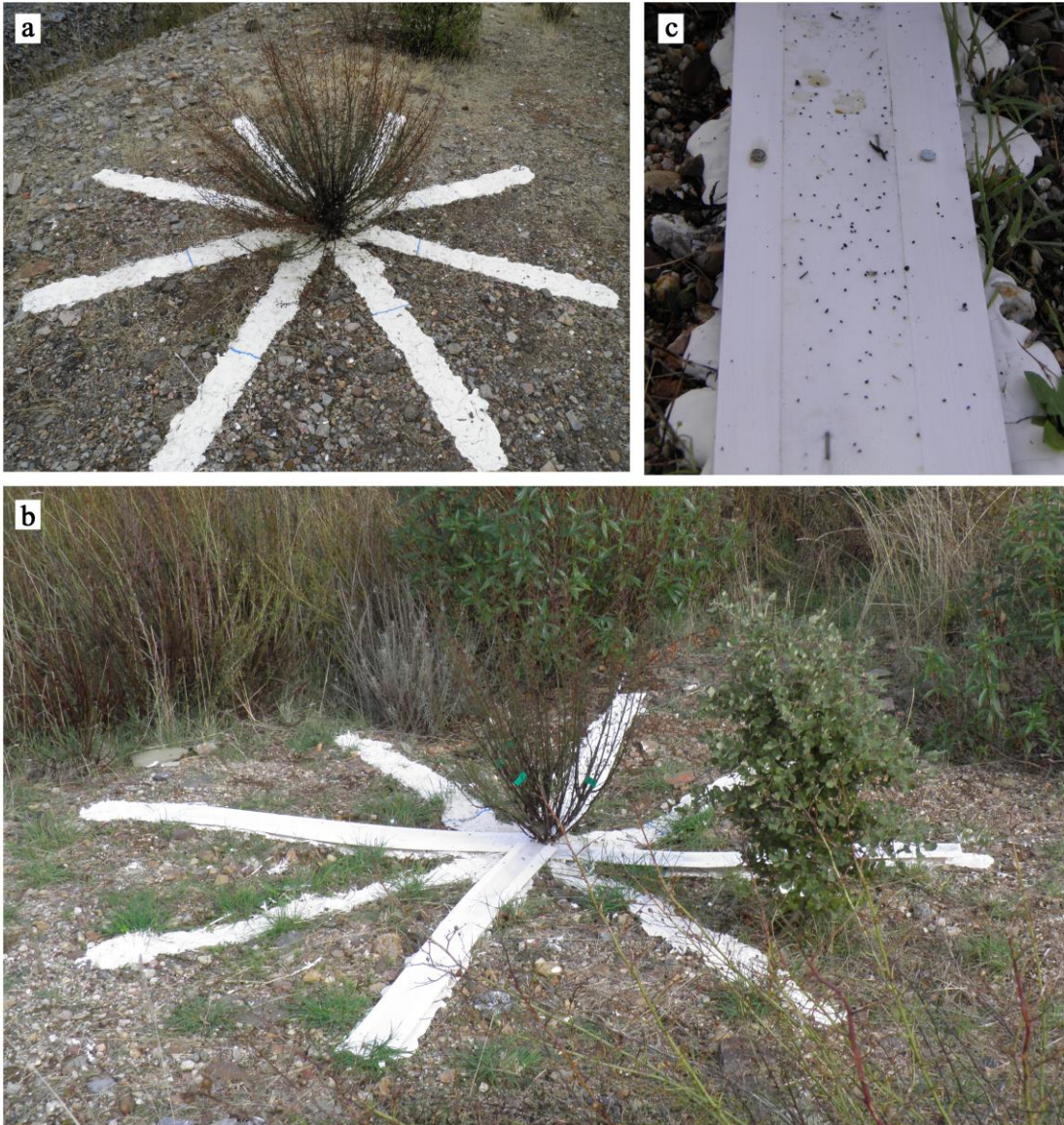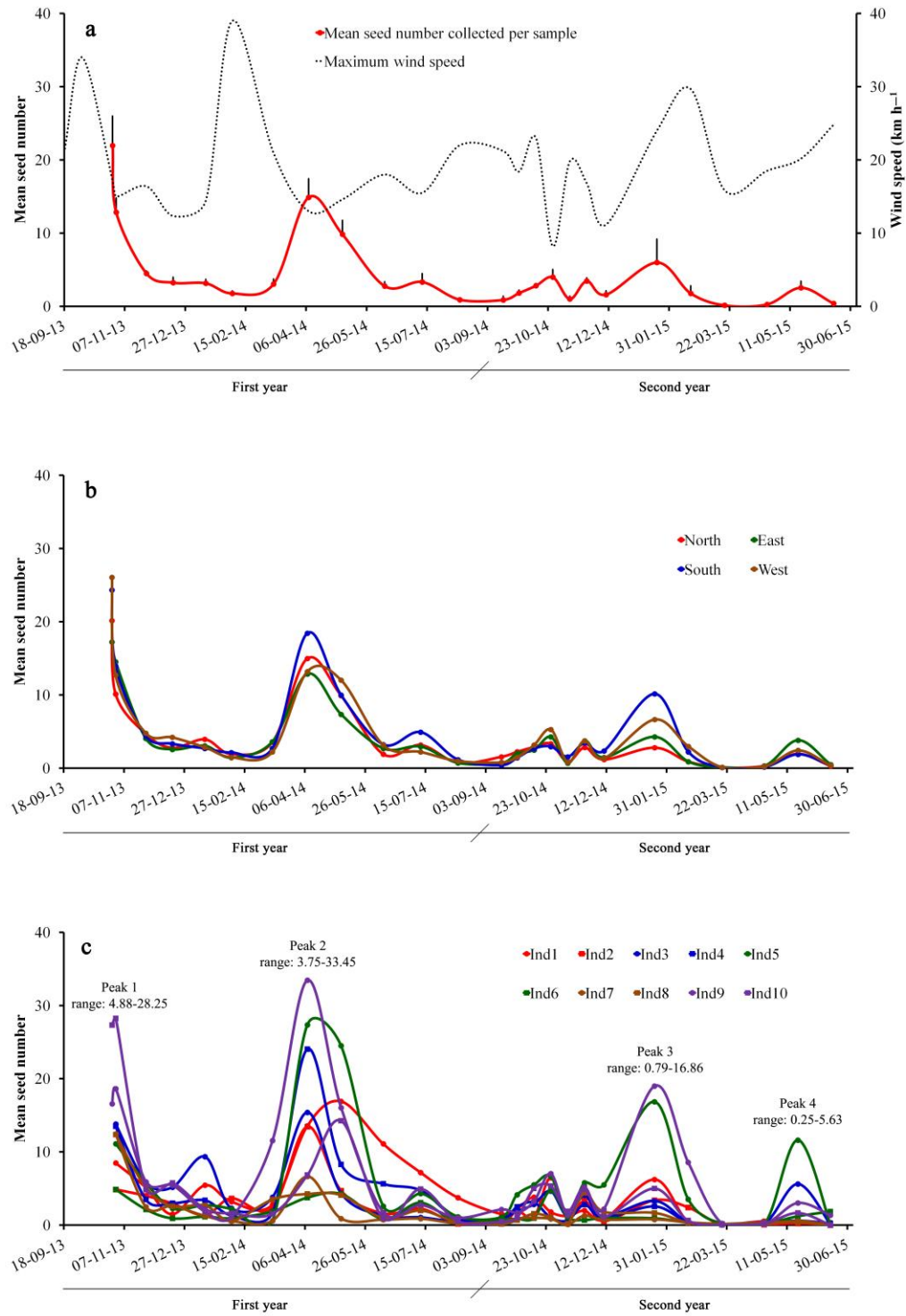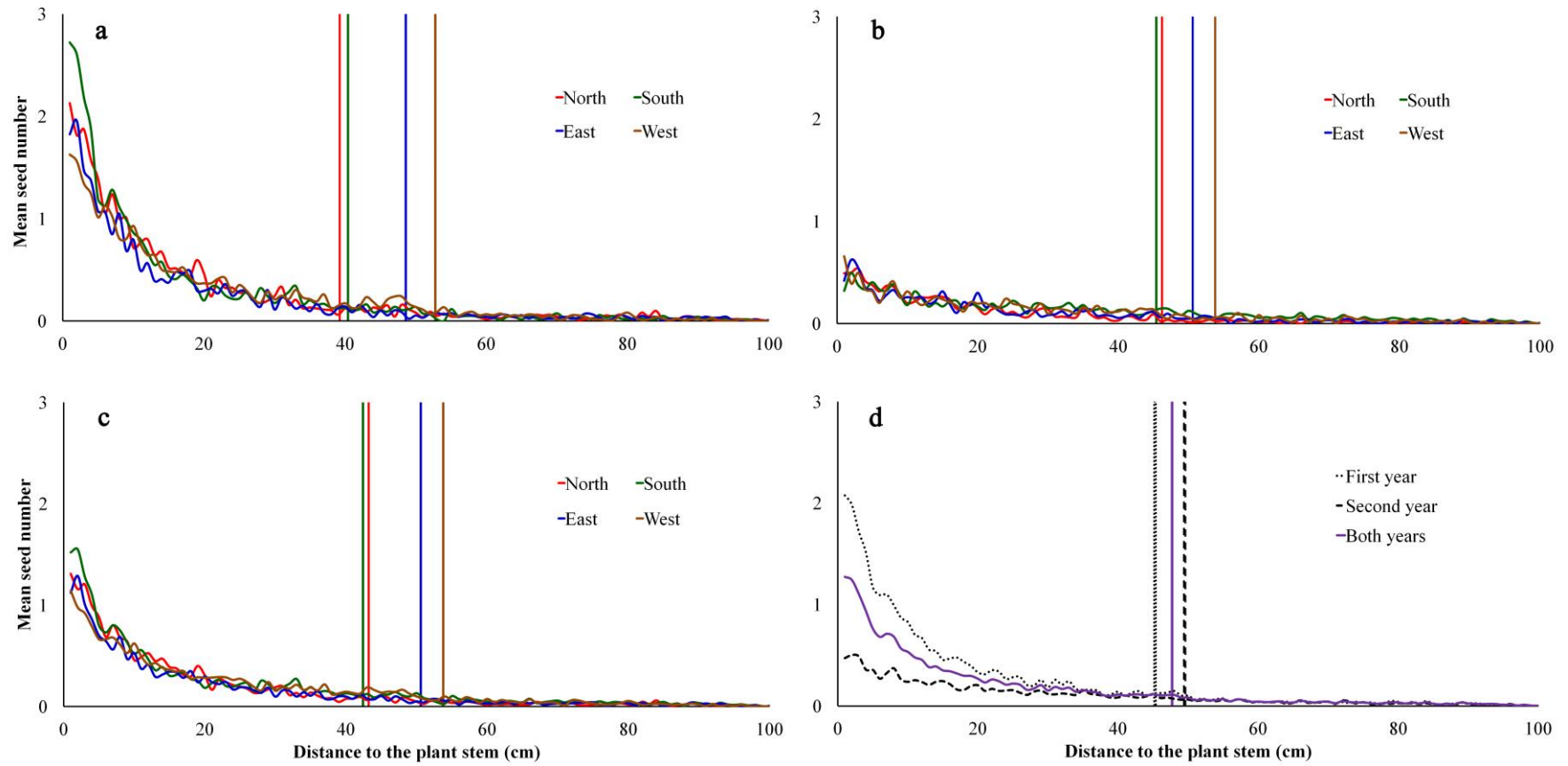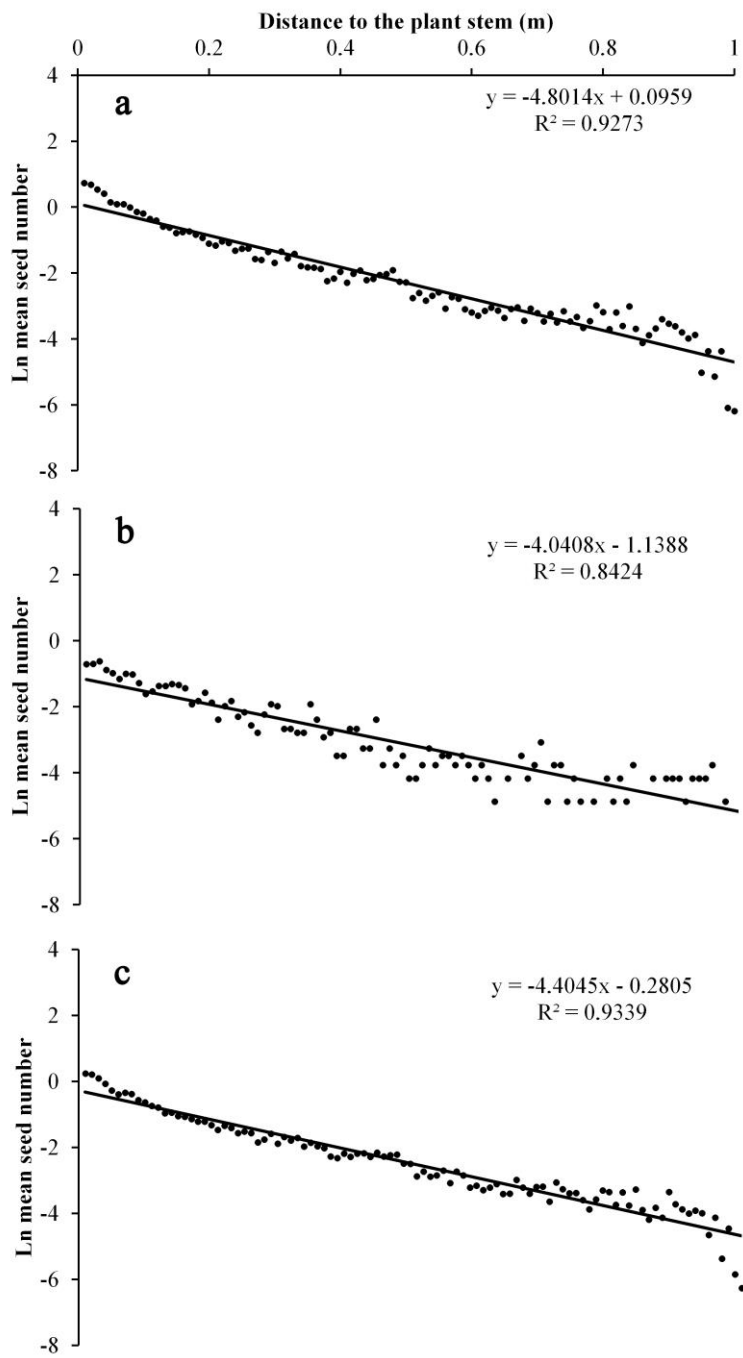
Figure 1

Figure 2

Figure 3

Figure 4

Figure 5
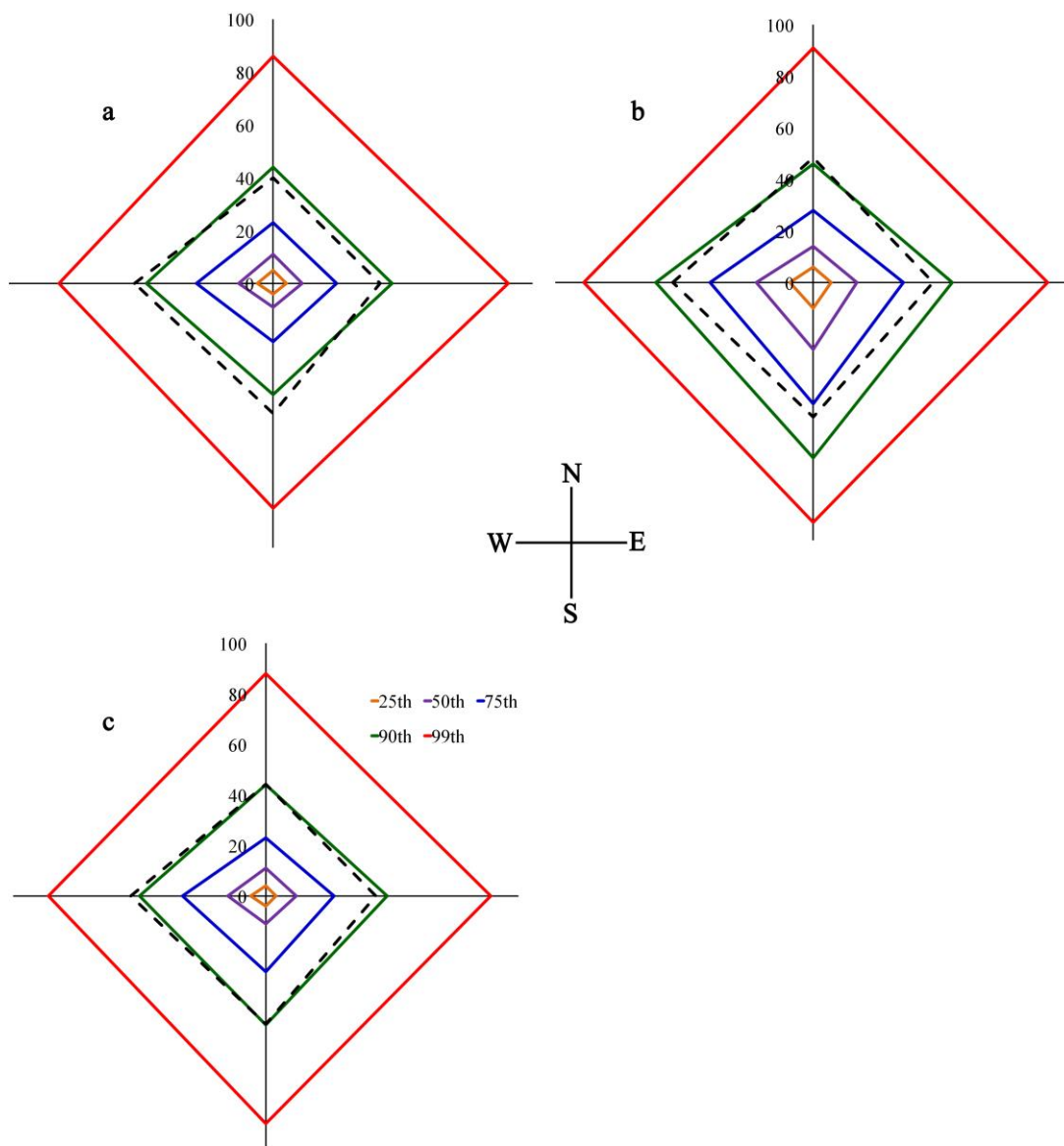
Table 1. Features (mean ± SD) of *Scrophularia canina* mother plants during the two studied years.

	First year	Second year	Statistical analysis between years	
Seed number/individual	32408.91 ± 16128.87	19341.07 ± 14677.18		**$F = 8.93, P = 0.015$**
Fruit number/individual	3341.13 ± 1662.77	1993.93 ± 1513.11		**$F = 8.93, P = 0.015$**
Fruit number/fruiting branch	81.60 ± 23.78	55.55 ± 20.63		**$F = 7.76, P = 0.021$**
Fruiting branch number/individual	41.00 ± 17.29	34.20 ± 19.05		**$F = 8.30, P = 0.018$**
Plant height (cm)	94.30 ± 9.21	84.50 ± 8.62		**$F = 6.40, P = 0.032$**
Plant lateral spread (cm)	45.54 ± 14.63	50.39 ± 12.58	Year	**$F = 8.84, P = 0.016$**
			Compass	**$F = 4.10, P = 0.016$**

All features, except plant lateral spread, were analysed by one-way repeated-measures ANOVA. Plant lateral spread was analysed by two-way repeated measures ANOVA.
Compass: principal compass directions.

Table 2. Repeated-measures ANOVA followed by application of the corrective Huynh-Feldt epsilon index to analyse temporal primary dispersal in *Scrophularia canina.*

	First year			Second year			Both years		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Census	3.778	**6.35**	**0.000**	4.701	**708.74**	**0.000**	3.849	**9.07**	**0.000**
Census × Individual	33.998	**3.95**	**0.000**	42.307	**99.12**	**0.000**	34.641	**4.45**	**0.000**
Census × Compass direction	3.778	0.51	0.720	4.701	**8.62**	**0.000**	3.849	0.53	0.707

Table 3. Generalized Estimating Equations (GEE) analysis of spatial primary seed dispersal fitted to a Gamma distribution.

	χ^2 Wald	df	*P*
Year	7.87	1	**0.005**
Compass direction	36.61	3	**0.000**
Distance to the plant stem	8.68×10^{14}	10	**0.000**
Year × Compass direction	42.49	3	**0.000**

Table 4. Statistical analysis of skewness and kurtosis indices, slope, modal dispersal distance, percentiles of the seed shadow, and seed percentage beneath plant lateral spread (% Seed).

	Year		Compass		Year × Compass	
	F / Wald	*P*	*F / Wald*	*P*	*F / Wald*	*P*
Skewness*	1.70	0.224	0.52	0.673	1.25	0.317
Kurtosis*	1.80	0.212	0.40	0.754	1.37	0.273
Slope*	**41.40**	**0.000**	1.55	0.225	0.33	0.808
Modal	**11.06**	**0.001**	7.80	0.050	**14.87**	**0.002**
Percentiles						
25th*	**27.37**	**0.000**	5.90	0.117	1.99	0.574
50th	**5.97**	**0.015**	**10.00**	**0.019**	**8.05**	**0.045**
75th	**6.60**	**0.010**	**24.36**	**0.000**	**17.17**	**0.001**
90th	**4.97**	**0.026**	5.51	0.138	**28.01**	**0.000**
99th*	3.59	0.058	**30.92**	**0.000**	1.16	0.764
% Seed	0.38	0.555	1.34	0.284	**4.28**	**0.013**

Compass: principal compass directions.
*: test values after model refitted without the not significant interaction.
Kurtosis: normalized by log (1-kurtosis)-transformation.
F (two-way repeated-measures ANOVA): skewness, kurtosis, slope and % seed.
Wald (GEE): modal and percentiles.

Table 5. Spearman correlations between different studied seed shadow traits and three plant traits by year and by combined years.

	First year			Second year			Both years		
	Height	Lateral spread	Seed production	Height	Lateral spread	Seed production	Height	Lateral spread	Seed production
Skewness	-0.191^{ns}	-0.061^{ns}	-0.227^{ns}	0.210^{ns}	$\mathbf{-0.387^{*}}$	-0.014^{ns}	0.141^{ns}	$\mathbf{-0.286^{*}}$	0.086^{ns}
Kurtosis	-0.135^{ns}	-0.076^{ns}	-0.201^{ns}	0.137^{ns}	$\mathbf{-0.323^{*}}$	-0.007^{ns}	0.127^{ns}	$\mathbf{-0.262^{*}}$	0.069^{ns}
Slope	0.100^{ns}	-0.083^{ns}	-0.103^{ns}	0.017^{ns}	0.057^{ns}	-0.195^{ns}	-0.207^{ns}	0.104^{ns}	$\mathbf{-0.521^{***}}$
Modal	0.083^{ns}	0.254^{ns}	0.112^{ns}	-0.021^{ns}	0.156^{ns}	-0.271^{ns}	-0.162^{ns}	$\mathbf{0.225^{*}}$	$\mathbf{-0.346^{**}}$
Percentiles									
25th	0.262^{ns}	$\mathbf{0.335^{*}}$	-0.130^{ns}	-0.167^{ns}	$\mathbf{0.560^{**}}$	-0.142^{ns}	-0.138^{ns}	$\mathbf{0.506^{***}}$	$\mathbf{-0.313^{**}}$
50th	$\mathbf{0.322^{*}}$	$\mathbf{0.312^{*}}$	-0.225^{ns}	-0.209^{ns}	$\mathbf{0.542^{***}}$	-0.111^{ns}	-0.119^{ns}	$\mathbf{0.479^{***}}$	$\mathbf{-0.303^{**}}$
75th	$\mathbf{0.372^{*}}$	$\mathbf{0.462^{**}}$	-0.183^{ns}	-0.249^{ns}	$\mathbf{0.547^{***}}$	-0.122^{ns}	-0.094^{ns}	$\mathbf{0.547^{***}}$	-0.202^{ns}
90th	0.177^{ns}	0.208^{ns}	-0.137^{ns}	-0.137^{ns}	0.054^{ns}	-0.027^{ns}	-0.094^{ns}	0.186^{ns}	-0.212^{ns}
99th	0.031^{ns}	-0.141^{ns}	-0.139^{ns}	-0.300^{ns}	0.175^{ns}	-0.206^{ns}	$\mathbf{-0.249^{*}}$	0.061^{ns}	$\mathbf{-0.310^{**}}$
% Seed	$\mathbf{0.363^{*}}$	$\mathbf{0.644^{***}}$	0.012^{ns}	-0.05^{ns}	$\mathbf{0.557^{***}}$	0.101^{ns}	0.158^{ns}	$\mathbf{0.559^{***}}$	0.111^{ns}

% Seed: percentage of seeds beneath plant's lateral spread. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant.