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RESEARCH

Autumn *Leucojum autumnale* and spring *L. trichophyllum*: the same flower type for different seasons with a different pollination scenario

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Abstract

Plant reproduction may experience distinct evolutionary and ecological dynamics depending on their flowering phenology. Consequently, floral traits might undergo divergence selection to adapt to the difference in the abiotic and the biotic environment, especially if these traits affect intensity of competition plants experience due to pollinators. The present study analyzed the plant floral display, floral rewards, and the pollination and breeding systems of two related species of *Leucojum*, the autumn-blooming *L. autumnale* and the spring-blooming *L. trichophyllum*. The aim of the current study is to compare reproductive aspects of *L. autumnale* and *L. trichophyllum* due to differing environmental conditions. Both species needed pollinators to reproduce sexually, with zero (*L. trichophyllum*) or low values (c. 7.7%; *L. autumnale*) for fructification after self-pollination vs. over 90% after natural pollination (both species). Their flowers opened in the morning and closed at night, and neither produced nectar, only pollen as reward to pollinators. Plants of the *L. autumnale* studied population produced 1–4 inflorescences per plant vs. only 1 in *L. trichophyllum*, and the former presented a greater density of flowers per surface unit. *Leucojum autumnale* flowers were visited by Hymenoptera and those of *L. trichophyllum* only by two Coleoptera, which were most abundant at sunrise and sunset, whereas in the middle of the day, they visited Cistaceae flowers. This latter occurrence may represent a temporal niche partitioning rather than competition in relation to visiting hours, as the *L. trichophyllum* flowers offer a place for insects to spend the night, a period in which the flowers of the Cistaceae species have lost their petals.

Keywords Breeding system · Floral density · Flowering phenology · Plant–pollinator interactions · Rhythm of flower opening

Introduction

Flowering phenology is generally phylogenetically constrained, and there is a tendency for closely related species to share similar flowering patterns; this phenomenon is usually the product of evolutionarily conserved adaptations to environmental conditions (Bolmgren and Cowan 2008; Davies et al. 2013), as occurs, for example, among the species of *Cyclamen* subgenus *Psilanthum* (Debussche et al. 2004). However, in some monophyletic groups, there are divergences in flowering time with different causes resulting in the non-random migration of genes that determine flowering time (Stam 1983), such as environmental disturbance. Indeed, flowering phenology is sensitive to some environmental cues (e.g., temperature, moisture, and light) that may exert selective pressure for genetic changes and adaptive evolution (Sherry et al. 2007). Divergences could also be a selection against hybrids by means of prezygotic barriers, which can result in instantaneous reproductive isolation among sympatric species (Dobzhansky 1940; Petit et al. 1997; Anacker and Strauss 2014) and, consequently, the reduced likelihood of mating (Martin and Willis 2007).

Differences in flowering phenology between related taxa could influence their reproductive biology, as phenology is one of the main driving forces of plant–pollinator networks (Morente-López et al. 2018). In effect, the switch in the flowering phenology can be accompanied by changes in the environment surrounding the flowering of the species;

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in this way, changes in pollinator diversity and blooming plant density and diversity might variously affect the reproductive success of species that bloom in different seasons (Dafni 1996; Debussche et al. 2004; Hegland and Boeke 2006; Albor et al. 2019), leading to the development of different adaptive strategies. In plants, it is known that ecological, geographical, and phenological differences, floral isolation by pollinators, and pollen precedence represent strong prezygotic barriers; however, there are also postzygotic barriers (e.g., fruit and seed set, lower hybrid viability, etc.) that have recently been shown to be considerable (Jiménez-López et al. 2023). Both barrier types may depend on genotype–environment interactions and genetic incompatibilities (revision in Jiménez-López et al. 2023). Although the pollinators as selective forces may mold flowering times, this is not always the case. According to Eisen et al. (2020), community context is likely to affect selection mediated by resource competition in many systems. Intraspecific competition at high floral densities and interspecific competition at low floral densities may be key species interactions among co-occurring plants that affect selection in floral traits (Eisen et al. 2020).

The present work is focused on two species of *Leucojum* L., a genus of monocotyledonous plants belonging to the family Amaryllidaceae that comprises polycarpic bulbous species with inflorescences in umbels (sometimes reduced to a flower) on long scapes. The genus comprises c. 10 species (Stern 1956; Meerow and Snijman 1998; Lledó et al. 2004) distributed across Europe and North Africa (Lledó et al. 2004). It includes several examples of related taxa that differ in the flowering period, such as *L. vernum* L., which blooms in late winter to early spring, and *L. aestivum* L., which blooms in spring, or at infra-specific level in the latter, with the subsp. *aestivum* blooming a few weeks later than the subsp. *pulchellum* (Salisb.) Briq. (Parolo et al. 2011). This is also the case with *L. autumnale* L. (=*Acis autumnalis* (L.) Sweet) and *L. trichophyllum* Schousb. (=*Acis trichophylla* (Schousb.) G.Don), two closely related species belonging to the monophyletic *Leucojum* subgenus *Acis* (Salisb.) Baker (Lledó et al. 2004). Both are Mediterranean species with overlapping distribution, from Portugal to the Atlantic coast of Morocco and North Africa (Lledó et al. 2004; Aedo 2013).

In the Mediterranean region, most geophyte species avoid the hot dry summer season and the cold winter through dormancy, and many of them bloom in autumn or winter while others bloom in spring (Dafni 1996; Debussche et al. 2004). During the autumn, when the blooming of *L. autumnale* occurs, there are few species blooming, so the competition for pollinators is low, as is the probability of pollen transfer among different species (Dafni 1996). While in spring, when *L. trichophyllum* blooms, the diversity of co-blooming species is high, and competition for pollinators could be expected to be greater (Hegland 2014; Albor et al. 2019). In these conditions, co-occurring species tend to avoid competition through niche partitioning by using different pollination resources or by deploying them at different times (Fründ et al. 2011; Pauw 2013; CaraDonna and Waser 2020).

Knowledge of the reproductive biology of the species of the genus *Leucojum* is very scarce, restricted mainly to *L. vernum* and *L. aestivum* (Percival 1955; Sprengel 1996; Parolo et al. 2011; Abeli et al. 2016; Wisdom et al. 2019; Maggi et al. 2021). The present study examined different aspects of the reproductive biology of the autumn-blooming *L. autumnale* and spring-blooming *L. trichophyllum* that share a distribution area in the southwest of the Iberian Peninsula. For both species, nothing is known about their floral biology (e.g., advertisement and rewards), or their pollination or reproduction systems. With this background, our main goal was to discover differences in features of the floral and reproductive biology between both species, including traits that may affect the attractiveness and accessibility of flowers to pollinators, as well as types of visiting insects and their flower visitation patterns. Once the differences between both species had been detected, our challenge was to investigate the consequences of the two sister species' flowering in different seasons for the pollination system and reproductive characteristics since, in autumn Mediterranean conditions, there is less diversity and lower density in blooming plants and active pollinators than in spring (Dafni 1996; Debussche et al. 2004). Furthermore, interpopulation variation in the level of rewards, pollinator types, and behavior and/or reproductive success within the same species is known in many cases (e.g., Proctor et al. 1996; Cosacov et al. 2008; Nadia and Machado 2014; Braunschmid et al. 2017). For this reason, we studied several zones (populations) for *Leucojum trichophyllum*.

Materials and methods

Species and populations studied

The flowers of *Leucojum* species open sequentially and remain open for several days, with some flowers of different ages opening at the same time. They are actinomorphic and nutant, and have six free white tepals, six stamens that dehisce by terminal pores, and a tricarpellary syncarpous pistil with a filiform style that produces a capsule (Stern 1956; Meerow and Snijman 1998; Lledó et al. 2004).

According to Aedo (2013), *L. autumnale* and *L. trichophyllum* have white flowers and differ in tepal size, longer in *L. trichophyllum*, and in the spathe, which is divided at the base in this species. Their stamens are similar, but the ovary is bigger in *L. trichophyllum* than in *L. autumnale*, and the seeds are larger in the latter species. In addition, the two lack green spots at the apex of the tepals.

Leucojum autumnale blooms mainly from July to November and is present in sandy acid soils in the southwest of the Iberian Peninsula, the Balearic Islands, Sardinia, Morocco, and Algeria, while *L. trichophyllum,* blooming mainly in March and April, is endemic to the southwest of the Iberian Peninsula and Morocco (Aedo 2013), being a characteristic species of the Malcolmietalia communities that inhabit deep sandy soils and coastal or continental paleodunes in the western Mediterranean (Rivas-Martínez et al. 2002). Known as snowflakes, they are often grown in gardens for the beauty and delicacy of their flowers.

The study was performed in Huelva province (southwestern Spain, Andalusia) (Online Resource 1). The populations studied were chosen within protected areas to achieve a greater degree of isolation from human activity and a better state of conservation of their natural conditions. Only one population of *L. autumnale* could be studied due to the lower frequency it occurs in sand systems of the protected areas of the coast of Huelva (López-Albacete 2009). The population was located in the Puntales zone (37°14′58,32"N 7° 0′59.61"O) (Fig. 1a), at the Odiel Marshes Natural Park, in an area of sand–clay soils populated by an open forest of *Pinus pinea* L. with a thicket of bushes of the *Asparago-Rhamnetum oleoidis* Rivas Goday community. For *L. trichophyllum*, three zones in the Doñana Natural Park were studied in order to discover potential interpopulation variation in the different aspects studied, all with sandy soils and populated by an open forest of *P. pinea* and covered by shrubland vegetation of the *Halimio halimifolii-Stauracanthetum genistoidis* Rivas Martínez & al. community (Rivas-Martínez et al. 1980): Laguna de los Cinco Pinos (henceforth CP) (37°9′10.65"N 6°47′19.69"O) (Fig. 1b), Tres Rayas (TR) (37°9′44.50"N 6°45′1.28"O), and Laguna de la Vaca (LV) (37°7′58.34"N 6°43′0.33"O).

During the study period, few species were in bloom in the *L. autumnale* population (October 2010) coinciding partially or totally with it, only one herbaceous species with conspicuous and colored corolla was in bloom, *Narcissus serotinus* L., as well as one entomophilous scrub, *Ulex argenteus* Welw. ex Webb. By contrast, we found more than 20 species of herbaceous plants in the three areas of *L. trichophyllum* studied (March and April 2010) with conspicuous and colored corollas flowering at the same time as *L. trichophyllum*, of which the most abundant in coverage were *Erodium aethiopicum* (Lam.) Brumh. & Thell., *Malcolmia triloba* (L.) Spreng., *Linaria spartea* (L.) Willd., and *Andryala arenaria* (DC.) Boiss. & Reuter; in addition, there were more than 10 species of entomophilous scrubs, including some species of Cistaceae, such as *Halimium halimifolium* (L.) Willk., *Halimium calycinum* (L.) K.Koch, *Cistus libanotis* L., and *Cistus salviifolius* L.

Fig. 1 Population of *Leucojum autumnale* (**a**) and CP population of *L. trichophyllum* (**b**)

Plant floral display

First, we took data on floral attributes of the populations and plants that could influence floral advertisement, since they could be modified due to differences in surrounding conditions (e.g., competition for pollinators with other co-occurring plants). From 20 plants of *L. autumnale* and 161 plants of *L. trichophyllum* (65 from CP, 32 from TR, and 64 from LV) randomly chosen, we obtained the following data: (a) number of inflorescences per plant; (b) number of flowers per inflorescence; (c) number of flowers per plant; and (d) inflorescence height (cm), measured from the apex of the bulb to the base of the umbel. In addition, distance to nearest individual of the same species (m) as a measure of plant density was studied. The density of blooming plants m^{-2} was calculated based on the ratio between distance to the nearest plant and density according to Clark and Evans (1954). As some parameters might be related to the size of the bulb, as a reserve organ, we also measured the bulb diameter at its widest part (mm).

Flower diurnal opening behavior is frequent in other *Leucojum* species, such as *L. aestivum* (Parolo et al. 2011), so another important trait in floral display was considered to be flower size in the populations at different times of the day, which could relate to floral attractiveness and accessibility. In two *L. trichopyllum* zones on two consecutive sunny days (March 31 and April 1, 2010), we measured the length of tepals and the diameter of the open flowers in the populations at different times, from sunrise to sunset, taking 637 measurements in different flowers (384 in CP and 253 in TR). For *L. autumnale*, we took the same measurements on a sunny day (October 28, 2010), taking 107 measurements. For both species, we measured the temperature in the population at each sampling time using a thermometer (AZ8908, AZ Instrument, Taichung City, Taiwan).

Floral rewards, and pollination and breeding systems

The main rewards sought by pollinators when visiting flowers of most angiosperms are nectar and pollen. To estimate the nectar production per flower on two sunny days for each species, and from two zones in the case of *L. trichophyllum* (CP and TR), we collected each time 25 entire plants that were conserved for 24 h in closed plastic bags at laboratory temperature (around 20 ºC) (Herrera 1985; Wyatt et al. 1992; Hidalgo and Cabezudo 1995). Then, we placed a graded 10-µm micropipette at the base of the stamens to collect the possible production of nectar by capillarity under a stereomicroscope.

Pollen:ovule ratio (P/O) is a general indicator of breeding system, which increases with the likelihood of cross-pollination, from cleistogamous to xenogamous species (Cruden 1977). To estimate the P/O ratio, flower buds were collected from the *L. autumnale* population and from the TR zone of *L. trichophyllum* and fixed in 70% ethanol–acetic acid (3:1). In the laboratory, 10 flowers of each species were manipulated under a stereomicroscope, separating one anther from each one, and the pistil. Each anther was macerated with a glass rod in 1 ml of soapy water in a 10-ml tube, and five samples of 10 µl were taken and deposited on a slide for microscope observation, counting the number of pollen grains in each sample. With the mean of the five samples, we estimated the pollen production in each anther, and by multiplying it by six, we obtained the estimated number of pollen grains in each of the 10 flowers. In each pistil, the number of ovules was extracted and counted. Then, we calculated the P/O ratio in each flower, dividing the average number of pollen grains by the number of ovules, and the mean and standard error were calculated for each species.

Rates of fruit set in self- and open pollination give information about the need for pollinators and the pollination rate under natural conditions, respectively (Dafni 1992). To estimate the fruit set under open pollination conditions, in the same 20 plants of *L. autumnale* and 161 of *L. trichophyllum* used for plant floral display measures, which had been exposed to pollinators during anthesis, we picked their fruits when ripe and before seed dispersal. The distance to the nearest individual of the same species was also measured. In the laboratory, for each flower, we counted the number of seeds per fruit, the number of inflorescences per plant, the order of the inflorescence in the plant (according to the sequence of anthesis), the height of the inflorescence, the number of flowers per inflorescence and per plant, and measured the diameter of the bulb. By dividing the number of fruits with seeds by the number of flowers, we estimated the fruit set percentages.

To estimate the fruit set under self-pollination conditions, nine plants of *L. autumnale* and 11 of *L. trichophyllum* (six from TR and five from LV) were bagged from before anthesis to flower senescence with a 1-mm plastic mesh cylinder (Online Resource 2a), whose rigidity avoided any rubbing against the flowers. Fruits were collected from all the plants when ripe and before seed dispersal, and in the laboratory, we counted the number of seeds per fruit for each flower. By dividing the number of fruits with seeds by the number of flowers, we estimated the fruit set percentages.

To evaluate whether the lower level of fruit set after self-pollination was the result of the failure of pollen grains to reach and germinate on the stigma surface, the dry stigmas of self-pollinated flowers and those from open pollination of both species were macerated in a lactophenol cotton blue solution on a slide for some hours. Then, they were observed under light microscopy after a gentle squash pressing with the cover glass, and the pollen grains attached to the stigma were counted (Online Resource 2b).

During the years in which we studied the flora and vegetation of aeolian sand systems of Doñana National Park (López-Albacete 2009), we visited many populations of *L. trichophyllum*. Two things caught our attention: (1) the lack of flying insects visiting the flowers of this species, although they did visit the flowers of other species in the plant communities, and (2) the frequent presence of beetles inside the flowers. However, when inspecting populations of *L. autumnalis* in other areas of the province of Huelva, we observed the presence of bees visiting its flowers. To verify these preliminary unaccounted for observations, two types of censuses were performed in both species to establish the identity, abundance, and behavior of pollinators: (a) one designed for flying pollinators, giving the results in percentage of flowers visited per hour, and (b) another designed to detect the temporary permanence of an insect with lower mobility inside the flowers, giving the results in individuals per flower. In *L. autumnale*, we made censuses for flying pollinators on a sunny day (October 28, 2010): at 10:00, 13:30, 14:00, 15:00, and 16:30 solar hours; in *L. trichophyllum*, we made censuses for flying pollinators at different times during the day between 7:00 and 19:00 solar time, in sunny days on March 30, 31 and April 1, 2010, in two of the zones studied (CP and TR). In each census, we observed a delimited surface of the population that exhibited near to 100 flowers in anthesis, and for 30 min, we annotated the type and number of pollinators, the number of flowers visited on that surface, as well as measuring temperature using the previously indicated thermometer. The overall time expended on these censuses was 2.5 h for *L. autumnale* and 5.5 h for *L. trichophyllum* (3.0 h in CP and 2.5 h in TR); nevertheless, in the case of *L. trichophyllum*, the results are based on a greater number of hours of observation of flying insects, since it would include the total time spent in the populations to make other measurements or observations.

In both species and on the same days, after we finished each census for flying pollinators, we performed a census to determine the presence of insects with lower mobility inside the flowers. We observed in an instant each flower individually and annotated the type and number of visitors, and counted the opened flowers in the inflorescence, the position of the flower in the inflorescence with respect to the number of opened flowers, the distance to the nearest plant with opened flowers, and temperature. The overall number of flowers observed was 157 in *L. autumnale* and 1087 in *L. trichophyllum* (763 in CP and 324 in TR).

Data analysis

All the statistical analyses were carried out using STATISTICA 8.0 (StatSoft Inc., USA), applying a significance level (α) of 0.05. Deviations from the arithmetic mean were calculated as standard error. Normality and homoscedasticity were tested using the Kolmogorov–Smirnov and the Levene tests, respectively. Comparison of means of data between both species was done using ANOVA (bulb diameter) or Mann–Whitney tests (distance among plants, number of inflorescences per plant, number of flowers per inflorescence, number of flowers per plant, inflorescence height, tepals length, and flower diameter). Comparison of data from the zones studied in *L. trichophyllum* was done with the ANOVA (bulb diameter and inflorescence height), Kruskal–Wallis (distance among plants, number of flowers per inflorescence, and inflorescence height) or Mann–Whitney tests (tepals length and flower diameter).

Generalized linear model (GLM) analyses fitted to a Poisson distribution with a logarithmic link function were performed to determine the influence of time and temperature, and zone in the case of *L. trichophyllum*, on flower diameter. For the two species, GLM analysis (Poisson distribution with a logarithmic link function) was also used to check the relative influence on the number of seeds per fruit of the distance from the plant to the nearest plant, bulb diameter, height of the inflorescence, number of flowers per inflorescence, and position of the flower in the inflorescence; in addition, for *L. autumnale*, the influence of the number of inflorescences, order of the inflorescence, and number of flowers per plant were also considered.

The relative influence on the number of insects with lower mobility inside the flowers recorded in *L. trichophyllum,* of the number of opened flowers per inflorescence, its flower position in the inflorescence with respect to the number of opened flowers, the distance to the nearest plant with opened flowers, and of the temperature, were also studied using a GLM analysis (Poisson distribution with a logarithmic link function).

Results

Plant floral display

Figures 2 and 3 and Online Resource 3 show the results obtained for plant traits in both species and in the three studied zones for *L. trichophyllum*. Plants of the *L. autumnale* studied population were closer together than plants of *L. trichophyllum* (Mann–Whitney test: U = 3.86, $p = 0.0000$)

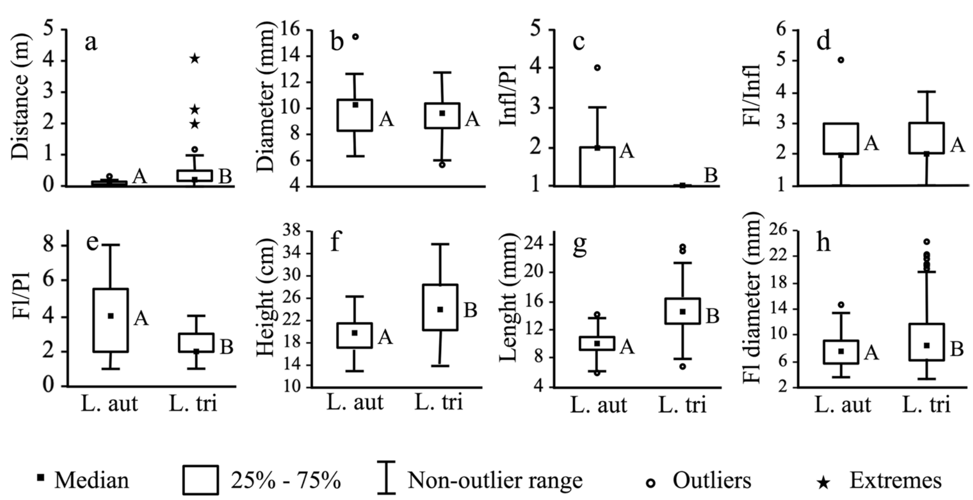

Fig. 2 Box plots for the plant parameters studied in the populations of *Leucojum autumnale* (L. aut) and *L. trichophyllum* (L. tri): distance to the nearest plant (**a**); bulb diameter (**b**); number of inflorescences per plant (**c**); number of flowers per inflorescence (**d**); number of flowers per plant (**e**); inflorescence height (**f**); tepals length (**g**); and flower diameter (**h**). Different letters beside the median in both species show significant differences between them ($p < 0.05$)

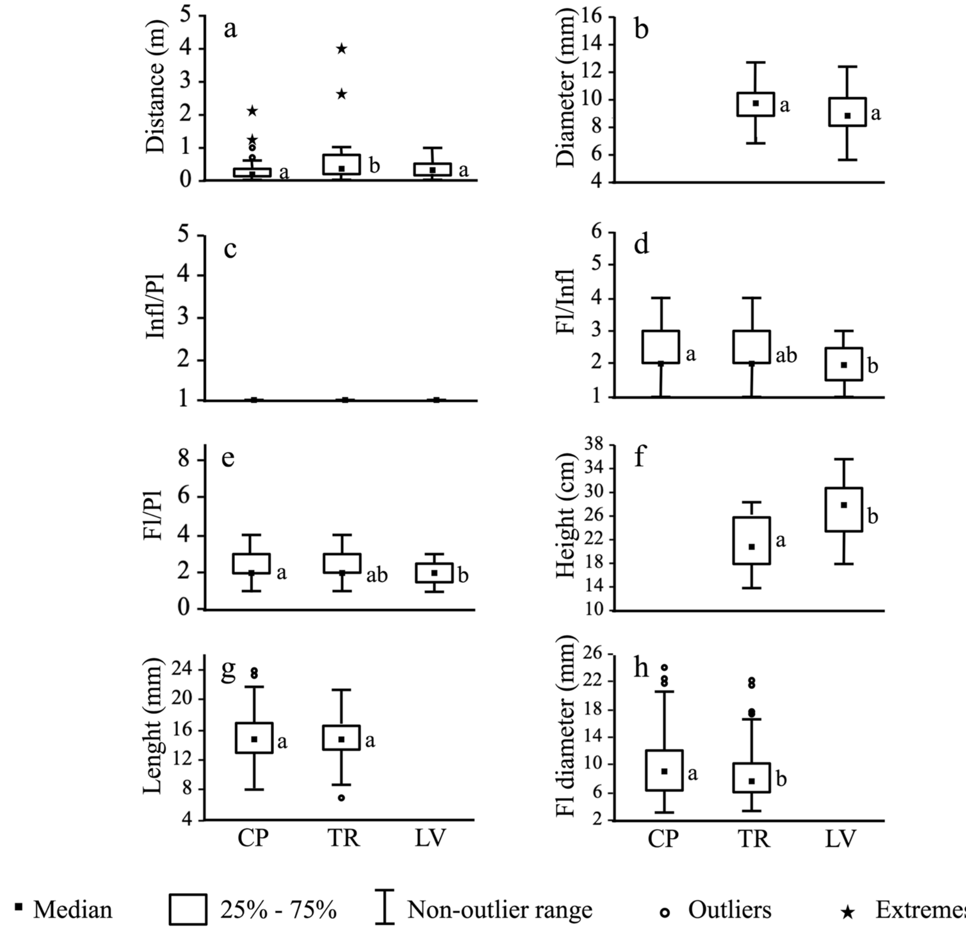

Fig. 3 Box plots for the plant parameters studied in the three zones (CP, TR, and LV) for *Leucojum trichophyllum*: distance to the nearest plant (**a**); bulb diameter (**b**); number of inflorescences per plant (**c**); number of flowers per inflorescence (**d**); number of flowers per plant (**e**); inflorescence height (**f**); tepals length (**g**); and flower diameter (**h**). Different letters beside the median in the three zones indicate significant differences among them ($p < 0.05$)

(Fig. 2a), and there were significant differences among *L. trichophyllum* zones (Kruskal–Wallis test: $H_{(2,160)} = 12.11$, $p = 0.0023$) (Fig. 3a). There were no significant differences in bulb diameter either between species (ANOVA test: $F = 3.25$, $p = 0.2940$) (Fig. 2b) or among *L. trichophyllum* zones (ANOVA test: $F = 1.96$, $p = 0.1660$) (Fig. 3b). The number of inflorescences per plant was always 1 in *L. trichophyllum* (Fig. 3c), and it was significantly greater in *L. autumnale* (Mann–Whitney test: $U = 563.5$, $p = 0.0000$), ranging from 1 to 4 (Fig. 2c). The number of flowers per inflorescence showed no significant differences between both species (Mann–Whitney test: $U = 2217.5$, $p = 0.8919$) (Fig. 2d) but it differed significantly among the zones of *L. trichophyllum* (Kruskal–Wallis test: $H_{(2,161)} = 11.43$, $p = 0.0033$) (Fig. 3d). The number of flowers per plant was bigger in *L. autumnale* than in *L. trichophyllum* (Mann–Whitney test: $U = 718.5$, $p = 0.0000$) (Fig. 2e), and, as occurred in the number of flowers per inflorescence, it differed significantly among the zones of *L. trichophyllum* (Fig. 3e). Inflorescences of *L. trichophyllum* were significantly taller than inflorescences of *L. autumnale* (Mann–Whitney test: $U = 414$, $p = 0.0000$) (Fig. 2f) and significantly different in their studied zones (ANOVA test: $F = 33.16$, $p = 0.0000$) (Fig. 3f).

Flowers of *L. trichophyllum* had longer tepals (Mann–Whitney test: $U = 3824.5$, $p = 0.0000$) (Fig. 2g) and greater diameter (Mann–Whitney test: $U = 27\ 020$, $p = 0.0006$) (Fig. 2h) than flowers of *L. autumnale* (Fig. 3g), and there were significant differences in the flower diameter in the two studied zones for *L. trichophyllum* (Mann–Whitney test: $U = 38\ 330$, $p = 0.0000$) (Fig. 3h), but not in their tepal length (Mann–Whitney test: $U = 36\ 778.5$, $p = 0.8044$) (Fig. 3g). Although the flowers of both species are white, they sometimes present pink tones toward the base.

Table 1 shows the results of the GLM analyses performed to test the effect of temperature, solar time, and zone in the case of *L. trichophyllum*, on the diameter of the open flower in populations. Flower diameter was affected by temperature and time of day, and by zone in the case of *L. trichophyllum*. Figure 4 shows the patterns of changes in flower diameter and temperature during day hours for both species, and for the two studied zones for *L. trichophyllum*, showing that flower diameter was smaller at night, with a diameter less than 6 mm in the case of *L. autumnale*, and below 5 mm in *L. trichophyllum*. Their diameters grew in the morning peaking early in the afternoon, almost 10 mm in *L. autumnale* and near 12 mm in *L. trichophyllum*, after which flower

Table 1 Generalized linear model results testing the effect of temperature, solar time, and zone, in the case of *Leucojum trichophyllum*, on flower diameter in the studied populations of *L. autumnale* and *L. trichophyllum*

	d.f	Wald statistic	*p*	Estimated coefficient	Standard error
L. autumnale					
Intercept	1	120.41	0.0000	1.4539	0.1325
Temperature	1	49.32	**0.0000**	**0.0494**	**0.0070**
Solar hour	1	7.21	**0.0073**	**−0.0324**	**0.0121**
L. trichophyllum					
Intercept	1	511.64	0.0000	1.5851	0.0700
Temperature	1	243.26	**0.0000**	**0.0407**	**0.0026**
Solar hour	1	40.99	**0.0000**	**−0.0271**	**0.0042**
Zone	1	30.21	**0.0000**	**0.0742**	**0.0135**

Significant predictive factors are indicated in bold ($p<0.05$)

diameter decreased. These patterns were very similar to those observed for changes in temperature throughout the day.

Floral rewards, and pollination and breeding systems

No nectar production and no scent were observed in the two species. Pollen production per flower was significantly greater (ANOVA test: F = 23.62, $p=0.0000$) in *L. trichophyllum* (120 372.00 ± 9399.45) than in *L. autumnale* (68 076.00 ± 53 235.94), and the number of ovules per flower was also significantly greater (Mann–Whitney test: U = 0.00, $p=0.0000$) in *L. trichophyllum* (24.90 ± 0.90) than in *L. autumnale* (16.00 ± 0.21).

The P/O ratio in *L. autumnale* was 4274.40 ± 349.95 and in *L. trichophyllum* 4913.07 ± 424.61, with no significant differences between them (ANOVA test: F = 1.35, $p=0.2610$).

The fruit set figure in open-pollinated flowers was over 90% in both species (Table 2), with the number of seeds

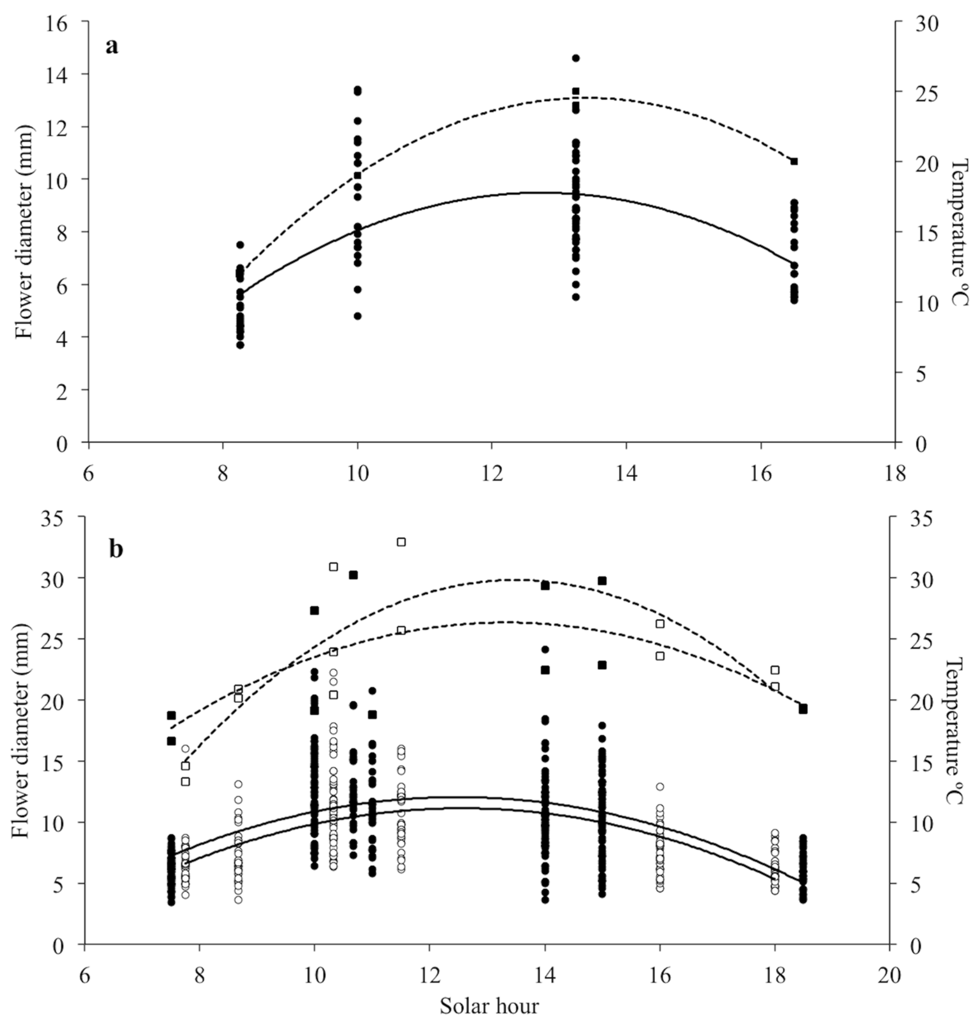

Fig. 4 Patterns of flower diameter changes (circles) and patterns of temperatures changes (squares) during day hours in both studied species: *Leucojum autumnale* (**a**) and *L. trichophyllum* (**b**). For *L. trichophyllum*, data from the two studied zones are presented: CP (black circles and squares) and TR (white circles and squares). For all data series, adjusted polynomial curves are represented, solid line for flower diameter and dashed line for temperature

Table 2 Number of studied plants, number of studied flowers per plant (with range), percentage of fruit set and number of seeds per fruit in open- and self-pollinated flowers in *Leucojum autumnale* and *L. trichophyllum*, and in the three zones studied (CP, TR, and LV) for *L. trichophyllum* in self-pollinated flowers, the results of the number of pollen grains counted on stigmas are presented

	Nº plants	Studied flowers/plant	% fruit set (n = nº fl)	Seeds/fruit (n = nº fr)	Pollen grains/stigma (n = nº stig)
L. autumnale Open pollination	20	2.60 ± 0.58 (1–6)	100.00 (n = 52)	12.79 ± 0.48 (n = 52)	
Self-pollination	9	2.89 ± 0.33 (1–6)	7.69 (n = 26)	5.50 ± 0.5 (n = 2)	6.39 ± 3.19 (n = 23)
L. trichophyllum Open pollination	160	2.25 ± 0.06 (1–4)	92.46 (n = 358)	17.41 ± 0.50 (n = 331)	
Self-pollination	11	2.00 ± 0.19 (1–3)	0.00 (n = 22)		0.68 ± 0.40 (n = 22)
CP Open pollination	64	2.45 ± 0.09 (1–4)	89.17 (n = 157)	18.33 ± 0.78 (n = 140)	
TR Open pollination	32	2.34 ± 0.12 (1–4)	95.95 (n = 75)	17.33 ± 1.30 (n = 71)	
LV Open pollination	64	2.00 ± 0.09 (1–3)	94.49 (n = 127)	16.39 ± 0.70 (n = 120)	
TR Self-pollination	6	2.00 ± 0.26 (1–3)	0.00 (n = 12)		0.58 ± 0.36 (n = 12)
LV Self-pollination	5	2.00 ± 0.32 (1–3)	0.00 (n = 10)		0.80 ± 0.80 (n = 10)

per fruit being significantly lower in *L. autumnale* than in *L. trichophyllum* (Mann–Whitney test: U = 5904.50, $p = 0.0003$); and there were no significant differences between the three studied zones for *L. trichophyllum* (Kruskal–Wallis test: $H_{(2, 331)} = 2.67$, $p = 0.2634$) (Table 2). In *L. autumnale*, the fruit set in self-pollinated flowers was 7.69%, and the only two fruits produced in these conditions had a lower number of seeds than fruits produced by open-pollinated flowers, but with no significant differences between them (Mann–Whitney test: U = 3.00, $p = 0.2410$). In self-pollinated flowers of *L. trichophyllum*, no fruit was produced in either of the two studied zones (Table 2). In both species, the number of pollen grains attached to stigmas in open-pollinated flowers was always too high to be counted (Online Resource 2b), while the number of pollen grains attached to stigmas in self-pollinated flowers of *L. autumnale* was 6.39 ± 3.19 vs. 0.68 ± 0.40 in self-pollinated flowers of *L. trichophyllum* (Table 2).

The results of the GLM analyses in *L. autumnale* (Table 3) showed that the only factor with a significant predictive effect on the number of seeds per fruit was flower position in the inflorescence, which showed a negative effect. For *L. trichophyllum*, GLM analyses showed that the factors with a significant predictive effect on the number of seeds per fruit of open-pollinated flowers were flower position in the inflorescence, with a negative effect,

Table 3 Generalized linear model results testing the effects of some parameters on the number of seeds per fruit for open-pollinated flowers in *Leucojum autumnale* and *L. trichophyllum*

L. autumnale	d.f	Wald statistic	*p*	Estimated coefficient	Standard error
Intercept	1	30.97	0.0000	2.3372	0.4200
Flower position	1	8.89	**0.0029**	**−0.1914**	**0.0642**
Bulb diameter	1	1.63	0.2024	0.0303	0.0238
Nº flowers/inflorescence	1	1.03	0.3097	0.0845	0.0832
Inflorescence position	1	0.81	0.3687	−0.0752	0.0836
Nº inflorescences/plant	1	0.09	0.7670	0.0363	0.1225
Distance to nearest plant	1	0.08	0.7816	−0.2169	0.7824
Nº flowers/plant	1	0.03	0.8721	0.0097	0.0603
Height of inflorescence	1	0.02	0.8924	0.0024	0.0178
L. trichophyllum	d.f	Wald statistic	*p*	Estimated coefficient	Standard error
Intercept	1	257.12	0.0000	2.5324	0.1579
Flower position	1	126.63	**0.0000**	**−0.3740**	**0.0332**
Height of inflorescence	1	11.07	**0.0009**	**0.0181**	**0.0054**
Nº flowers/inflorescence	1	8.93	**0.0028**	**0.1068**	**0.0357**
Zone	1	3.42	0.0645	0.0561	0.0303
Bulb diameter	1	1.26	0.2617	0.0185	0.0165
Distance to nearest plant	1	0.00	0.9972	−0.0001	0.0273

Significant predictive factors are indicated in bold ($p < 0.05$)

and height of the inflorescence and number of flowers per inflorescence, both with a positive effect (Table 3).

Regarding floral visitors, all were insects that contacted the sexual organs of the flowers and are hereafter referred to as pollinators; in addition, our results should be taken as preliminary due to the limited time dedicated to the censuses. In *L. autumnale*, only a few Hymenoptera were observed (only *Megachile sp.* was recognized), and no other insect was seen to alight on in any of the 157 flowers observed. Hymenoptera individuals were observed mainly in the early morning, with an estimated 23.08% of flowers visited per hour^{-1} at 10:00 h, 1.92% at 16:30 h, and 0% at 13:30 h, 14:00 h, and 15:00 h. However, some Hymenoptera pollinators were observed at the same times in the flowers of *Narcissus serotinus* L. that coexisted in the *L. autumnale* population. Temperatures registered during the sampling day are presented in Fig. 4.

In *L. trichophyllum*, no insect was observed flying among its flowers during the 5.5 h of observation. However, two kinds of Coleoptera were observed inside the flowers. The most abundant was *Lobonyx aeneus* (Fabricius 1787) (Online Resource 2c), both males and females, which appeared at frequencies of 0.1193 ± 0.0136 individuals per flower^{-1} in the CP zone (91 individuals in 763 flowers), and of 0.0123 ± 0.0061 per flower^{-1} in the TR zone (four individuals in 763 flowers), with significant differences in their abundance between zones (Mann–Whitney test: U = 112 154, $p = 0.0000$). The other beetle observed inside the flowers was an *Attagenus* species, which appeared at frequencies of 0.0328 ± 0.0077 per flower^{-1} in the CP zone (four individuals in 324 flowers) and of 0.0031 ± 0.0031 per flower^{-1} in the TR zone (one individual in 324 flowers), with significant differences in their abundance between zones (Mann–Whitney test: U = 120 584, $p = 0.0089$).

In terms of the presence of *L. aeneus*, the results of the GLM analyses (Table 4) showed that all the factors tested presented a significant predictive value: number of opened flowers; distance to the nearest plant with opened flowers and the zone studied, with a positive effect; and temperature and flower position, with a negative effect. In relation to the presence of *Attagenus sp.*, the results of the GLM analyses (Table 4) showed that only three factors had significant predictive values: distance to the nearest plant and the number of opened flowers, with a positive effect; and the flower position, with a negative effect.

As temperature had a significant negative effect on the presence of *Lobonyx aeneus*, and temperature varies throughout the day (Fig. 4), the mean and standard error of the abundance of both insects are presented in Fig. 5, grouped in four time intervals.

Discussion

Our results indicate that the flowers of the two studied *Leucojum* species belonging to the subgenus *Acis* (=*Acis* Salisb.) only show pollen as reward and not nectar, and present similar floral phenotypes (same shape, white—sometimes pink at the base—, scentless, and pendulous). In addition, the populations of the autumnal species showed an aggregated spatial distribution pattern with a higher density of smaller flowers per square meter vs. a spaced or uniform pattern with a lower density of larger flowers in the spring species. In addition, there was interpopulation variation in some floral and reproductive parameters of *L. trichophyllum*.

Table 4 Generalized linear model results testing the effects of some parameters on the frequency of *Lobonyx aeneus* and *Attagenus sp.* (Coleoptera) on flowers of *Leucojum trichophyllum*

Lobonyx aeneus	d.f	Wald statistic	*p*	Estimated coefficient	Standard error
Intercept	1	2.19	0.1388	−1.5832	1.0697
Opened flowers	1	27.69	**0.0000**	**0.9420**	**0.1790**
Distance to next plant	1	22.33	**0.0000**	**1.1184**	**0.2367**
Temperature	1	16.91	**0.0000**	**−0.1356**	**0.0330**
Flower position	1	14.30	**0.0002**	**−1.2972**	**0.3430**
Zone	1	4.72	**0.0297**	**1.6664**	**0.7670**
Attagenus sp.	d.f	Wald statistic	*p*	Estimated coefficient	Standard error
Intercept	1	0.28	0.5962	−8.6299	16.2958
Distance to next plant	1	34.56	**0.0000**	**1.3784**	**0.2345**
Opened flowers	1	19.78	**0.0000**	**1.0723**	**0.2411**
Flower position	1	5.65	**0.0175**	**−1.7840**	**0.7506**
Temperature	1	3.51	0.0612	0.0793	0.0424
Zone	1	0.03	0.8640	2.7779	16.2231

Significant predictive factors are indicated in bold ($p < 0.05$)

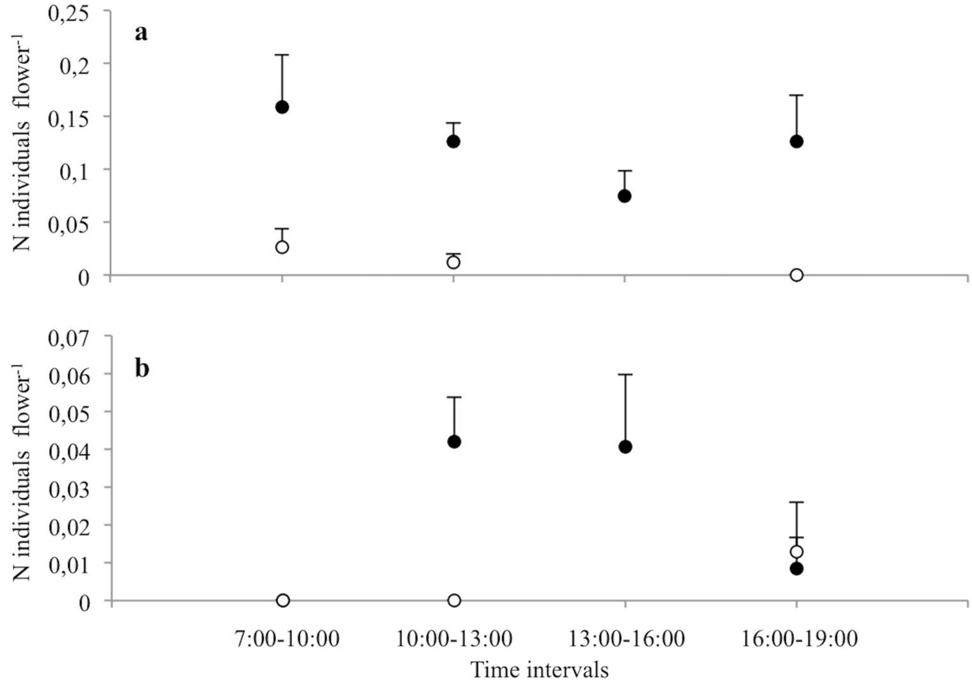

Fig. 5 Average and standard errors of the abundance (individuals per flower^{-1}) of *Lobonyx aeneus* **(a)** and *Attagenus sp.* **(b)** in flowers of *Leucojum trichophyllum* on CP (black circles) and TR (white circles) zones, in four time intervals

This is the first time that data on flower visitors of *L. autumnale* and *L. trichophyllum* have been recorded, which interact in different ways with other co-blooming species in the studied areas. Together, our results on the floral biology, pollination, and reproduction will contribute to improving knowledge of the genus, given the scarcity of such type of studies in *Leucojum*.

Plant floral display

Some features of the zones and plants studied showed differences between the two species. Factors in which there were no interspecific differences were the bulb diameter or the number of flowers per inflorescence. The diameters of the flowers of both species' populations were at their smallest between sunset and sunrise, growing in the morning, reaching maximum diameter in the early afternoon, after which they decreased again. A diurnal opening behavior is frequent in other *Leucojum* species, such as *L. aestivum*, which is also stimulated by temperature changes (Parolo et al. 2011). Although we have related the opening mechanism to intraday temperature variations, other environmental factors could affect it, such as dull weather (e.g., fog and rain), lighting, deep shade, etc., indicated for other species (e.g., Proctor et al. 1996; Ortega-Olivencia et al. 1995).

Nevertheless, the two species differed in some features that affect their advertisement (e.g., the number of flowers per plant, the flower size, the length of the inflorescence, and the density of blooming plants in their populations), some of which could be related to their co-blooming context with other species. *L. autumnale* produced almost double the number of flowers per plant, and their individuals were closer together than individuals of *L. trichophyllum*. The density of blooming plants m^{-2} was greater in *L. autumnale* (51 blooming plants m^{-2} in the studied population) than in *L. trichophyllum* (it ranged from 0.4 to 3 blooming plants m^{-2} in their studied zones). These results revealed an aggregated spatial distribution in *L. autumnale*, probably as a result of vegetative propagation, as occurs in other species of the genus (e.g., *L. aestivum*, Parolo et al. 2011) and/or a shorter distance seed dispersal system. In addition, the fact that *L. autumnale* produces several inflorescences per plant determines a greater density of flowers in its populations, which could play an important role in its reproductive success (Dafni 1996). In fact, it may be crucial to attracting more pollinators and to providing an accurate pollen flow in the autumnal low species richness environment, in which the activity of pollinators was positively predicted by blossom density (Hegland and Boeke 2006).

In *L. trichophyllum*, although with interpopulation variation, individuals were more distant from each other, which could indicate that sexual reproduction by seeds is the main reproduction mechanism in this species that only produce one inflorescence; this more separate spatial pattern could also be due to a longer-distance seed dispersal system. Note that its seeds are smaller than seeds of *L. autumnale* (Aedo 2013). Probably, the lower flower density could be related to the fact that in spring, when it blooms, there is a high amount of floral rewards in communities in which it lives, that could have a dilution effect on pollinator visitations, reducing the proportion of flowers visited (Veddeler et al. 2006; Hegland 2014). Besides, in such conditions, a high flower density might not be an advantage, while a lower density might reduce intraspecific competition for pollination resources and increase the probability of a flower being pollinated (Rathcke 1983; Dauber et al. 2010; Pauw 2013). The lower flower density could explain why *L. trichophyllum* resorts to other attributes to increase its advertisement, such as larger flowers and inflorescences taller than those of *L. autumnale*.

Floral rewards, and pollination and breeding systems

Neither of the two studied species produced scent, at least to the human nose, or nectar, the latter coinciding with the results from Hidalgo and Cabezudo (1995) for *L. autumnale*. The absence of nectar has also been observed in the spring *L. aestivum* (Percival 1955), but in case of spring snowflake *L. vernum*, natively widespread in central and northern Europe (Lledó et al. 2004), Sprengel (1996) described the presence of nectar and scent. Therefore, both *L. autumnale* and *L. trichophyllum* should be classified as pollen flowers.

Both species showed similar breeding systems, with pollinators being needed to produce an adequate level of fruit set. The two species showed a P/O ratio within the range of xenogamous species (Cruden 1977). Results concur with the fact that *L. trichophyllum* did not produce any fruit after self-pollination, and *L. autumnale* produced a lower fruit set percentage with a lower number of seeds in this condition. In *L. aestivum*, self-incompatibility (SI) has been stated (Knuth 1909; Parolo et al. 2011), and in another Amaryllidaceae species, a gametophytic SI system has been revealed (Streher et al. 2018). New future experiments may confirm the existence or not of SI systems in our species. In both species, there were very few pollen grains attached to the stigma by pollen tubes in self-pollinated flowers, so we assume that, apart from the possible existence of SI, the rate of spontaneous self-pollination is very low, probably due to the herkogamy, and to the opening of anthers through a terminal pore (Sprengel 1996; Parolo et al. 2011).

The studied populations of the two species showed good reproductive success in open-pollinated flowers with high fruit set percentages (over 90%) and a high average number of seeds per fruit, revealing that 80% of the ovules had been fertilized in *L. autumnale* and 70% in *L. trichophyllum*. The flower position in the inflorescence had a significant negative predictive effect on the number of seeds per fruit, which could be attributable to competition for resources among flowers opened at different times and/or to architectural effects (Diggle 1995; Medrano et al. 2000; Valtueña et al. 2012).

In *L. trichophyllum*, the number of flowers per inflorescence and the height of the inflorescence, which varied in terms of interpopulation, also had a positive predictive effect on the number of seeds per fruit. The first factor would relate to plant vigor, but the second would be more closely related to the advertisement of flowers to pollinators.

Neither of the species studied did seed production per fruit seem to relate to the density of plants, and so there seems to be no pollen limitation in the populations studied. However, in other *Leucojum* species, the seed set increased considerably with the density of blooming plants, and so their fertilization was pollen-limited in low-density populations (e.g., *L. aestivum*, Parolo et al. 2011; Abeli et al. 2016; and *L. nicaeense* Ardoino and *L. fabrei* Quézel & Girerd, Diadema et al. 2004).

Since there was no or very little self-pollination, the flow of pollen must be carried out through pollinators. Which pollinators acted on each of the species, and under what circumstances did they operate? In *L. autumnale*, only Hymenoptera, mainly *Megachile sp.*, were observed visiting their flowers, although the sample time was quite limited, and more studies are needed to be conclusive. Hymenoptera were described as visitors in other species of *Leucojum*, as in winter to early spring-blooming *L. vernum*, in which *Apis mellifera* Linnaeus (1758) was attracted by nectar guides and scent (Sprengel 1996). For *L. aestivum,* Parolo et al. (2011) included as potential pollinators Hymenoptera (e.g., bees and bumblebees), nocturnal and diurnal Lepidoptera, and observed that flowers functioned as shelters for several species of beetles and spiders. However, a study based on field observations by Maggi et al. (2021) identified 18 arthropod taxa carrying *L. aestivum* pollen, of which the taxon with the highest pollen load on its body was the *Lasioglossum punctatissimum* bee (Schenck 1853), and the most frequent visitor being the pollen-feeding beetle *Dasytes plumbeus* (Müller 1776).

Hymenoptera were registered in *L. autumnale* flowers mainly early in the morning and some late in the evening, but no during the middle hours of the day, even though at that time, Hymenoptera pollinators were observed to be active in the erect flowers of *Narcissus serotinus* L. cohabiting with *L. autumnale*. Similarly, in *Narcissus cavanillesii* A.Barra & G.López and *N. serotinus* species that cohabit with *L. autumnale*, Marques et al. (2007) observed in Portuguese communities that these species were visited by a small number of insects, including Hymenoptera, Diptera, and Coleoptera; in this case, *L. autumnale* competed with these *Narcissus* species for pollinators, but to a lesser degree, which is confirmed by our observations, at least for individual *Megachile*, because they visited the flowers of *L. autumnale* mainly in the morning, while we observed the activity of insects in flowers of *N. serotinus* throughout the day. In *N. serotinus*, nectar production is vestigial (Vicedo et al. 2018) and is known the presence of Lepidoptera visiting its flowers (Pérez Chiscano 1985; Marques et al. 2007); so, it offers pollinators both pollen and nectar as rewards, which could determine their preference for *N. serotinus* over *L. autumnale*, which only offers pollen. The higher frequency of visits to flowers of *L. autumnale* early in the morning is perhaps related to greater accessibility to pollen at this time, after the flower had remained closed and unvisited overnight.

Both during the present study, and in the previous years, in which many populations of *L. trichophyllum* were visited, and despite not recording quantifiable data, no flying insects were observed on its flowers, although some Coleoptera were present. This could be consistent with observations on *L. aestivum*, in which Percival (1955) and Wisdom et al. (2019) observed that honeybee pollination activity in its flowers was rare, probably by the lack of nectar as reward, absence of scent, and poor pollen production (Percival 1955; Parolo et al. 2011; Abeli et al. 2016; Maggi et al. 2021).

Two kinds of Coleoptera were seen alighting on the flowers of *L. trichophyllum*, *Lobonyx aeneus*, the most abundant, and *Attagenus sp.* Both insects were recorded in different abundance in the zones studied and probably used the flowers for feeding on pollen, for reproductive encounters, or as a refuge, and the flowers did not show symptoms of damage due to their presence. Obviously, with their movement inside the flower, they rub against the sexual organs, probably behaving as pollinators. The scant attraction of its flowers to honeybees, a frequent pollinator of other species of the plant community that bloomed at the same time (Herrera 1988), is reflected in the low frequency of its pollen in spring honeys from Spain (Ortiz and Fernández 1995) and Morocco (Terrab et al. 2001, 2002).

The presence of *L. aeneus* is encouraged firstly by the number of flowers opened in the inflorescence, possibly related to the attractiveness of the floral structure, and to the distance to the nearest plant with opened flowers, which means that the more isolated the plant, the more insect visits to its flowers occur; it could be the result of the uniform distribution of these insects and insect concentration in the flowers of lower plant density patches. However, the presence of *L. aeneus* was diminished by temperature, so it was less abundant around the hottest hours of the day while more frequent at sunrise and sunset; another factor was flower position, the presence of *L. aeneus* being more frequent in the first flowers of the inflorescence.

Lobonyx aeneus is common in the flowers of Cistaceae species that had a considerable presence in the communities studied (e.g., *Halimium halimifolium*, *H. calycinum*, *Cistus libanotis* L., and *C. salviifolius*); their blooming overlapped with that of *L. trichophyllum* in a major or minor period (Herrera 1986), and all of them represent important pollen rewards for pollinators (Herrera 1988). In *L. trichophyllum*, we estimated about 120 000 pollen grains per flower, which is greater than that recorded in the species of Cistaceae mentioned (e.g., *H. halimifolium*: 55 000 grains per flower; *H. calycinum*: 13 000 grains per flower; *C. libanotis*: 55 000 grains per flower; and *C. salviifolius*: 106 000 grains per flower; Herrera 1987, 1988). However, its grains are much smaller (Valdés et al. 1987) so the net mass of pollen is small, and therefore, it could be considered a poor pollen producer, as occurs with *L. aestivum* (Abeli et al. 2016; Maggi et al. 2021).

In those conditions, models established by Hanoteaux et al. (2013) predicted that the survival prospects of the less attractive species, in our case *L. trichophyllum* due to its flowers without nectar, with smaller size and lower pollen production (in mass), would improve when uniformly distributed and avoiding growing in clumps, because Cistaceae serves as a magnet species increasing local pollinator activity. This could explain the low densities of *L. trichophyllum* populations compared to populations of *L. autumnale*. Although the presence of a richly rewarding plant species may increase the rate of pollinator visitation over a less rewarding species, the beneficial effect may be counteracted by a detrimental increase in heterospecific pollen transfer (Thomson et al. 2019). Flowers of Cistaceae species open in the morning and their petals fall in the afternoon, so, the numerous Coleoptera that visit them must find other places to stay during the rest of the day. Thus, the presence of *Lobonyx aeneus* in the flowers of *L. trichophyllum* showed interpopulation variation; its maximum abundance at sunrise and sunset, coinciding with the absence of opened flowers in the Cistaceae, could mean that the presence of this pollinator in both taxa does not imply competition for its services but temporary cessation of its visit, turning interspecific competition into a temporal niche partitioning. It implies an increasing complementarity in high-blooming plant diversity in spring, ensuring stable provision of pollination services (Venjakob et al. 2016). Other spring species of *Leucojum* probably show a similar pollination strategy in their communities, as observed by Maggi et al. (2021), who found another Coleoptera (*Dasytes plumbeus*) in the flowers of *L. aestivum* in Italy and discovered differences interpopulation and temporal in beetle activity.

In the case of the presence of *Attagenus sp.*, the factors that affected its abundance were: distance to the next plant, the number of opened flowers, and the flower position, with a negative effect, all with the same sign as in *Lobonyx aeneus*, which could be explained in the same way, though data for *Attagenus sp.* did not show that its presence depended on temperature. Individual *Attagenus sp.* were also observed in the flowers of *Halimium calycinum* by Herrera (1988).

Conclusions

Our results showed autumn-blooming *L. autumnale* produced a greater density of flowers in its population while the spring-blooming *L. trichophyllum* zones displayed a lower density of flowers. The two species exhibit the same floral type, with the same daily schedule of anthesis, with no scent or nectar production, and with similar P/O ratios, and both needed the assistance of pollinators to fruit set.

We observed high reproductive success in the populations of both studied species in terms of fruit set and seed number per fruit, but using different pollinators. In the *L. autumnale* population, Hymenoptera activity was important mainly in the morning. In the *L. trichophyllum* zones, only Coleoptera were observed inside the flowers, with *Lobonyx aeneus* being the most frequent. Nevertheless, future studies are needed to confirm the existence or not of SI systems and the role of herkogamy to explain the low rates of fruit set after autonomous selfing. In the two species, further studies are needed to complete the role of different pollinators in relation to the neighboring blooms at each time.

Information on Electronic Supplementary Material

Online Resource 1. Map showing the location of the study zones.
Online Resource 2. a Plant of *Leucojum trichophyllum* bagged with a 1-mm plastic mesh cylinder; **b** stigma of an open-pollinated flower of L. trichophyllum in lactophenol cotton blue solution for pollen tubes observation; and **c** individuals of *Lobonyx aeneus* in a flower of *L. trichophyllum*.
Online Resource 3. Plant parameters studied in the populations of *Leucojum autumnale* and *L. trichophyllum*, as well as in the three zones (CP, TR, and LV) for *L. trichophyllum*.

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Declarations

Competing interests The authors declare no competing interests.

References

Abeli T, Cauzzi P, Rossi G, Adorni M, Vagge I, Parolo G, Orsenigo S (2016) Restoring population structure and dynamics in translocated species: learning from wild populations. Pl Ecol 217:183–192. https://doi.org/10.1007/s11258-015-0529-x

Aedo C (2013) *Leucojum* L. In: Rico E, Crespo MB, Quintanar A, Herrero A, Aedo C (eds) Flora iberica. Real Jardín Botánico, CSIC, Madrid, pp 328–335

Albor C, García-Franco JG, Parra-Tabla V, Díaz-Castelazo C, Arceo-Gómez G (2019) Taxonomic and functional diversity of the co-flowering community differentially affect *Cakile edentula* pollination at different spatial scales. J Ecol 107:2167–2181. https://doi.org/10.1111/1365-2745.13183

Anacker BL, Strauss SY (2014) The geography and ecology of plant speciation: range overlap and niche divergence in sister species. Proc Roy Soc B 281:20132980. https://doi.org/10.1098/rspb.2013.2980

Bolmgren KD, Cowan P (2008) Time–size tradeoffs: A phylogenetic comparative study of flowering time, plant height and seed mass in a north-temperate flora. Oikos 117:424–429. https://doi.org/10.1111/j.2007.0030-1299.16142.x

Braunschmid H, Mükisch B, Rupp T, Schäffler I, Zito P, Birtele D, Dötterl S (2017) Interpopulation variation in pollinators and floral scent of the lady's-slipper orchid *Cypripedium calceolus* L. Arthropod-Pl Interact 11:363–379. https://doi.org/10.1007/s11829-017-9512-x

CaraDonna PJ, Waser NM (2020) Temporal flexibility in the structure of plant–pollinator interaction networks. Oikos 129:1369–1380. https://doi.org/10.1111/oik.07526

Clark PJ, Evans FC (1954) Distance to nearest neighbor as a measure of spatial relationships in populations. Ecology 35:445–453

Cosacov A, Nattero J, Cocucci AA (2008) Variation of pollinator assemblages and pollen limitation in a locally specialized system: the oil-producing *Nierembergia linariifolia* (Solanaceae). Ann Bot (Oxford) 102:723–734. https://doi.org/10.1093/aob/mcn154

Cruden RW (1977) Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. Evolution 31:32–46. https://doi.org/10.2307/2407542

Dafni A (1992) Pollination ecology. A practical approach. Oxford University Press, Oxford

Dafni A (1996) Autumnal and winter pollination adaptations under Mediterranean conditions. Bocconea 5:171–181

Dauber J, Biesmeijer JC, Gabriel D, Kunin WE, Lamborn E, Meyer B, Nielsen A, Potts SG, Roberts SPM, Sõber V, Settele J, Steffan-Dewenter I, Stout JC, Teder T, Tscheulin T, Vivarelli D, Petanidou T (2010) Effects of patch size and density on flower visitation and seed set of wild plants: a pan-European approach. J Ecol 98:188–196. https://doi.org/10.1111/j.1365-2745.2009.01590.x

Davies TJ, Wolkovich EM, Kraft NJ, Salamin N, Allen JM, Ault TR, Betancourt JL, Bolmgren K, Cleland EE, Cook BI, Crimmins TM, Mazer SJ, McCabe GJ, Pau S, Regetz J, Schwartz MD, Travers SE (2013) Phylogenetic conservatism in plant phenology. J Ecol 101:1520–1530. https://doi.org/10.1111/1365-2745.12154

Debussche M, Garnier E, Thompson JD (2004) Exploring the causes of variation in phenology and morphology in Mediterranean geophytes: a genus-wide study of *Cyclamen*. Bot J Linn Soc 145:469–484. https://doi.org/10.1111/j.1095-8339.2004.00298.x

Diadema K, Médail F, Affre L, Castagné H, Torre F, Bretagnolle F (2004) Ecology and demography of two endangered narrow endemic plants (*Leucojum*, Amaryllidaceae) in southern France. Ecology, Conservation and Management of Mediterranean climate ecosystems. Millpress Science Publishers, Rotterdam, pp 1–9

Diggle PK (1995) Architectural effects and the interpretation of patterns of fruit and seed development. Annual Rev Ecol Syst 26:531–552. https://doi.org/10.1146/annurev.es.26.110195.002531

Dobzhansky T (1940) Speciation as a stage in evolutionary divergence. Amer Naturalist 74:312–321. https://doi.org/10.1086/280899

Eisen KE, Wruck AC, Geber MA (2020) Floral density and co-occurring congeners alter patterns of selection in annual plant communities. Evolution 74:1682–1698. https://doi.org/10.1111/evo.13960

Fründ J, Dormann CF, Tscharntke T (2011) Linné's floral clock is slow without pollinators–flower closure and plant-pollinator interaction webs. Ecol Lett 14:896–904. https://doi.org/10.1111/j.1461-0248.2011.01654.x

Hanoteaux S, Tielbörger K, Seifan M (2013) Effects of spatial patterns on the pollination success of a less attractive species. Oikos 122:867–880. https://doi.org/10.1111/j.1600-0706.2012.20801.x

Hegland SJ (2014) Floral neighbourhood effects on pollination success in red clover are scale-dependent. Funct Ecol 28:561–568. https://doi.org/10.1111/1365-2435.12223

Hegland SJ, Boeke L (2006) Relationships between the density and diversity of floral resources and flower visitor activity in a temperate grassland community. Ecol Entomol 31:532–538. https://doi.org/10.1111/j.1365-2311.2006.00812.x

Herrera J (1985) Nectar secretion patterns in southern Spanish Mediterranean scrublands. Israel J Bot 34:47–58. https://doi.org/10.1080/0021213X.1985.10677008

Herrera J (1986) Flowering and fruiting phenology in the coastal shrublands of Doñana, south Spain. Vegetatio 68:91–98. https://doi.org/10.1007/BF00045059

Herrera J (1987) Flower and fruit biology in southern Spanish Mediterranean shrublands. Ann Missouri Bot Gard 74:69–78. https://doi.org/10.2307/2399263

Herrera J (1988) Pollination relationships in southern Spanish Mediterranean shrublands. J Ecol 76:274–287. https://doi.org/10.2307/2260469

Hidalgo MI, Cabezudo B (1995) Producción de néctar en matorrales del sur de España (Andalucía). Acta Bot Malac 20:123–132. https://doi.org/10.24310/abm.v20i.8840

Jiménez-López FJ, Arista M, Talavera M, Cerdeira Morellato LP, Pannell JR, Viruel J, Ortiz Ballesteros PL (2023) Multiple pre- and postzygotic components of reproductive isolation between two co-occurring *Lysimachia* species. New Phytol 238:874–887. https://doi.org/10.1111/nph.18767

Knuth P (1909) Handbook of Flower Pollination: Based Upon Hermann Müller's Work 'The Fertilisation of Flowers by Insects'. Clarendon Press, Oxford

Lledó MD, Davis AP, Crespo MB, Chase MW, Fay MF (2004) Phylogenetic analysis of *Leucojum* and *Galanthus* (Amaryllidaceae) based on plastid *mat*K and nuclear ribosomal spacer (ITS) DNA sequences and morphology. Pl Syst Evol 246:223–243. https://doi.org/10.1007/s00606-004-0152-0

López-Albacete I (2009) Flora and vegetation of Aeolian sand systems of Doñana. PhD Thesis, University of Huelva, Huelva. https://rabida.uhu.es/dspace/handle/10272/685

Maggi LA, Abeli T, Rossi G, Gobbi M (2021) Flower-visiting and pollen-carrying arthropods of *Leucojum aestivum* L. (Amaryllidaceae) in wild, reintroduced and ex situ populations. Pl Ecol 222:965–975. https://doi.org/10.1007/s11258-021-01154-0

Marques I, Rosselló-Graell A, Draper D, Iriondo JM (2007) Pollination ecology and hybridization between *Narcissus cavanillesii* A. Barra & G. López and *N. serotinus* L. in Portugal. Bocconea 21:65–75

Martin NH, Willis JH (2007) Ecological divergence associated with mating system causes nearly complete reproductive isolation between sympatric *Mimulus* species. Evolution 61:68–82. https://doi.org/10.1111/j.1558-5646.2007.00006.x

Medrano MN, Guitián P, Guitián J (2000) Patterns of fruit and seed set within inflorescences of *Pancratium maritimum* (Amaryllidaceae): nonuniform pollination, resource limitation, or architectural effects? Amer J Bot 87:493–501. https://doi.org/10.2307/2656592

Meerow AW, Snijman DA (1998) Amaryllidaceae. In: Kubitzki K (ed) Flowering plants, monocotyledons: Lilianae (except Orchidaceae). Springer, Berlin, pp 83–110

Morente-López J, Lara-Romero C, Ornosa C, Iriondo JM (2018) Phenology drives species interactions and modularity in a plant-flower visitor network. Sci Rep 8:1–11. https://doi.org/10.1038/s41598-018-27725-2

Nadia TL, Machado IC (2014) Interpopulation variation in the sexual and pollination systems of two Combretaceae species in Brazilian mangroves. Aquatic Bot 114:35–42. https://doi.org/10.1016/j.aquabot.2013.12.003

Ortega-Olivencia A, Carrasco JP, Devesa JA (1995) Floral and reproductive biology of *Drosophyllum lusitanicum* (L.) Link (Droseraceae). Bot J Linn Soc 118:331–351

Ortiz PL, Fernández I (1995) Contribución al conocimiento melitopalinológico de Huelva y Sevilla. Acta Bot Malac 20:97–105

Parolo G, Abeli T, Rossi G, Dowgiallo G, Matthies D (2011) Biological flora of Central Europe: *Leucojum aestivum* L. Perspect Pl Ecol Evol 13:319–330. https://doi.org/10.1016/J.PPEES.2011.05.004

Pauw A (2013) Can pollination niches facilitate plant coexistence? Trends Ecol Evol 28:30–37. https://doi.org/10.1016/j.tree.2012.07.019

Percival MS (1955) The presentation of pollen in certain angiosperms and its collection by *Apis mellifera*. New Phytol 54:353–368

Pérez Chiscano JL (1985) Insectos polinizantes en *Narcissus serotinus* L. Fontqueria 8:5–8

Petit C, Lesbros P, Ge X, Thompson JD (1997) Variation in flowering phenology and selfing rate across a contact zone between diploid and tetraploid *Arrhenatherum elatius* (Poaceae). Heredity 79:31–40. https://doi.org/10.1038/hdy.1997.120

Proctor M, Yeo P, Lack A (1996) The Natural History of Pollination. Harper Collins Publishers, London

Rathcke BJ (1983) Competition and facilitation among plants for pollination. In: Real L (ed) Pollination Biology. Academic Press, Orlando, pp 305–329

Rivas-Martínez S, Costa M, Castroviejo S, Valdés E (1980) Vegetación de Doñana (Huelva, España). Lazaroa 2:5–190

Rivas-Martínez S, Díaz TE, Fernández-González F, Izco J, Loidi J, Lousã M, Penas A (2002) Vascular plant communities of Spain and Portugal (addenda to the syntaxonomical checklist of 2001, part I). Itinera Geobot 15:5–922

Sherry RA, Zhou X, Gu S, Arnone JA III, Schimel DS, Verburg PS, Wallace LL, Luo Y (2007) Divergence of reproductive phenology under climate warming. Proc Natl Acad Sci USA 104:198–202. https://doi.org/10.1073/pnas.0605642104

Sprengel CK (1996) Discovery of the secret of nature in the structure and fertilization of flowers. In: Lloyd DG, Barrett CH (eds) Floral Biology. Springer, Boston, pp 3–43

Stam P (1983) The evolution of reproductive isolation in closely adjacent plant populations through differential flowering time. Heredity 50:105–118. https://doi.org/10.1038/hdy.1983.13

Stern FC (1956) Snowdrops and snowflakes. A study of the genera *Galanthus* and *Leucojum*. The Royal Horticultural Society, London

Streher NS, Guerra E, Lüdtke R, Semir J, Dutilh JHA (2018) Self-incompatibility in *Habranthus gracilifolius* (Amaryllidaceae): pre-and post-pollination barriers. Brazil J Bot 41:375–384. https://doi.org/10.1007/s40415-018-0463-y

Terrab A, Díez MJ, Valdés B (2001) Análisis polínico de mieles en las regiones de Ouazzane y costa Atlántica (noroeste de Marruecos). Acta Bot Malac 26:79–88. https://doi.org/10.24310/abm.v26i0.7401

Terrab A, Valdés B, Díez MJ (2002) Análisis polínico de mieles en la región del pre-Rif central (noroeste de Marruecos). Acta Bot Malac 27:65–74. https://doi.org/10.24310/abm.v27i0.7309

Thomson JD, Fung HF, Ogilvie JE (2019) Effects of spatial patterning of co-flowering plant species on pollination quantity and purity. Ann Bot (Oxford) 123:303–310. https://doi.org/10.1093/aob/mcy120

Valdés B, Díez MJ, Fernandez I (1987) Atlas polínico de Andalucía occidental. Instituto de Desarrollo Regional de la Universidad de Sevilla, Utrera

Valtueña FJ, Ortega-Olivencia A, Rodríguez-Riaño T (2012) Regulation of fruit and seed set in *Anagyris foetida* L. (Fabaceae): The role of intrinsic factors. Pl Biosyst 146:190–200. https://doi.org/10.1080/11263504.2012.679976

Veddeler D, Klein AM, Tscharntke T (2006) Contrasting responses of bee communities to coffee flowering at different spatial scales. Oikos 112:594–601. https://doi.org/10.1111/j.0030-1299.2006.14111.x

Venjakob C, Klein AM, Ebeling A, Tscharntke T, Scherber C (2016) Plant diversity increases spatio-temporal niche complementarity in plant-pollinator interactions. Ecol Evol 6:2249–2261. https://doi.org/10.1002/ece3.2026

Vicedo J, Draper Munt D, Marques I, Véla E (2018) *Narcissus serotinus*. The IUCN red list of threatened species 2018: e.T13147080A18613955. https://doi.org/10.2305/IUCN.UK.2018-1.RLTS.T13147080A18613955.en

Wisdom MM, Richardson MD, Karcher DE, Steinkraus DC, McDonald GV (2019) Flowering persistence and pollinator attraction of early-spring bulbs in warm-season lawns. HortScience 54:1853–1859. https://doi.org/10.21273/HORTSCI14259-19

Wyatt R, Broyles SB, Derda GS (1992) Environmental influences on nectar production in milkweeds (*Asclepias syriaca* and *A. exaltata*). Amer J Bot 79:636–642. https://doi.org/10.1002/j.1537-2197.1992.tb14605.x