

ARTICLE

Contributions to the knowledge of ecology and floral biology of *Ophrys* × *vasconica* (Orchidaceae) in one of the disjunct populations of central-western Iberian Peninsula (Spain)

Contribuciones al conocimiento de la ecología y biología floral de *Ophrys* × *vasconica* (Orchidaceae) en una de las poblaciones disyuntas de la Península Ibérica (España)

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Abstract: CONTRIBUTIONS TO THE KNOWLEDGE OF ECOLOGY AND FLORAL BIOLOGY OF *OPHRYS* × *VASCONICA* (ORCHIDACEAE) IN ONE OF THE DISJUNCT POPULATIONS OF CENTRAL-WESTERN IBERIAN PENINSULA (SPAIN). — The species *Ophrys* × *vasconica* (O. Danesch & E. Danesch) P. Delforge is a relatively understudied taxon with significant interest from the perspective of Iberian orchid flora. So far, there have been no specific studies conducted on the ecology and floral biology of the species. The results of this study show that *Pyrus bourgaeana* Decne. groves, where the orchid *O.* × *vasconica* lives, act as nurse plants. This means that the orchids are isolated from detrimental temporary waterlogging during the flowering season, as well as from herbivory caused by livestock. The results also show that the size of the floral spike decreases with distance from the nurse tree trunks. This translates into increased reproductive fitness the closer individuals are to the nurse trees, as individuals with longer floral spikes exhibited higher male pollination success (removal of at least one pollinia per floral spike). Although no pollinators were observed in action over flowers, there was fruit development, indicating high female pollination success.

Keywords: facilitation; mutualism; nurse plant; *Ophrys*; Orchidaceae.

Resumen: CONTRIBUCIONES AL CONOCIMIENTO DE LA ECOLOGÍA Y BIOLOGÍA FLORAL DE *OPHRYS* × *VASCONICA* (ORCHIDACEAE) EN UNA DE LAS POBLACIONES DISYUNTAS DEL CENTRO-OESTE DE LA PENÍNSULA IBÉRICA (ESPAÑA). — La especie *Ophrys* × *vasconica* (O. Danesch & E. Danesch) P. Delforge es un taxón relativamente poco estudiado que posee interés desde la perspectiva de la orquidoflora ibérica. Hasta la fecha, no se han realizado estudios específicos sobre la ecología y la biología floral de esta especie. Los resultados de este estudio muestran que los bosquetes de *Pyrus bourgaeana* Decne., donde vive la orquídea *O.* × *vasconica*, actúan como plantas nodrizas, de forma que las orquídeas quedan aisladas del encharcamiento temporal perjudicial durante la temporada de floración, así como de la herbivoría causada por el ganado presente en la zona. Los resultados también indican que el tamaño de la espiga floral disminuye a medida que aumenta la distancia desde los troncos de los árboles nodrizas. Esto se traduce en una mayor aptitud reproductiva cuanto más cercanas están las orquídeas a las plantas nodrizas, ya que los individuos con espigas florales más largas presentaron un mayor éxito de polinización masculina, expresado como probabilidad de polinización masculina (al menos un polinio retirado por espiga floral). Aunque no se observaron polinizadores en acción sobre las flores, se evidenció el desarrollo de frutos, lo que indica una alta tasa de polinización femenina efectiva.

Palabras clave: facilitación; mutualismo; *Ophrys*; Orchidaceae; planta nodriza.

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Supplementary information ↓

INTRODUCTION

The species *Ophrys* × *vasconica* (O. Danesch & E. Danesch) P. Delforge is a perennial geophyte endemic to the southern and southwestern regions of France and northern part of the Iberian Peninsula (Delforge, 2006). It represents a relatively understudied taxon with significant interest from the perspective of Iberian orchid flora. Authors such as Delforge (2006) have classified this species as ‘in process of formation’, as it emerges from the introgression of *O. dyris* Maire with *O. fusca* Link s.l. This ‘hybridization’ leads to shared characteristics with both parent species. Another noteworthy aspect about *O. × vasconica* is that its populations do not appear to be result of the same speciation event. This is evidenced by three disjunct populations in the central-western region (in Spain and Portugal) and an additional one in the southwestern part of the peninsula (in Portugal) (Rico Hernández, 1980; Lewis, 2009; Tyteca et al., 2017), despite being mainly restricted to the northern third of the Iberian Peninsula (Aldasoro & Sáez, 2005) and the southern and southwestern regions of France (Delforge, 2006). This limited geographic distribution qualifies it as a rather rare or scarce taxon in the Iberian Peninsula (Rabinowitz, 1981).

In addition to the scarce chorological data for this species, which are mostly published sporadically in regional flora or orchid flora works, there is also a lack of specific information regarding its population ecology and reproductive biology. A problem that also affects the clarification of its taxonomy. Several authors, such as Bernardos et al. (2003) and García-Barriuso et al. (2010), have conducted karyological analyses on this species, using samples collected from the Spanish population in the central-western part of the peninsula. However, when it comes to its species-level ecology, there are only brief comments available regarding the characteristics of the habitats in which it grows, as well as the accompanying flora present in those habitats. Therefore, no specific studies have examined the potential role of accompanying flora as facilitating nurse plants, which could influence population performance, as has been demonstrated for other terrestrial orchid species in Mediterranean ecosystems (Baldelomar et al., 2019; Atala et al., 2020).

On the other hand, and in a more extensive manner than in the case of genetics and ecology, experiments and field studies have been conducted to determine both the bee species involved in the pollination of the genus *Ophrys* L., as well as the mechanisms of specificity involved in this pollination (BorgKarlson, 1990; Vereecken & Schiestl, 2008; Paulus, 2006; 2017; 2018). The pollinators of *O. × vasconica* have been studied and identified in some of these works (Benito Ayuso, 2017; Paulus, 2017, 2018). However, there have been no studies conducted on the floral biology of the species, nor on the rates of pollination and fruiting in its populations.

Specific studies on the ecology and biology of the species, such as those presented here, can significantly enhance understanding of its ecological and biological characteristics. We examined one of the three disjunct population in the central-western part of the Iberian Peninsula during the years 2017 and 2018. More specifically, the following aspects were investigated: a) population size; b) the nurse role of tree vegetation on *O. × vasconica*; c) the relationships between floral spike size and the number of open flowers, as well as reproductive success (pollination probability); and d) the rates of pollination and fruiting in the population.

MATERIAL AND METHODS

Study area

The study was conducted in central-western Iberian Peninsula (Fig. 1A), involving one of the three disjunct *Ophrys × vasconica* populations from the main distribution area (Rico Hernández, 1980; Delforge, 1995; Hermosilla, 1998, 2001; Benito Ayuso *et al.*, 1999; Segura Zubizarreta *et al.*, 2000; Lizaur & Lazare, 2004; Lewis, 2009; García-Barriuso *et al.*, 2010; Lowe & Tyteca, 2012; Alejandro Sáenz *et al.*, 2014; Paulus, 2017; Ruiz de Gopegui & García Cayón, 2017; Tyteca *et al.*, 2017; Hernández Varas, 2021). The study population is located near the town of Saelices el Chico (latitude 40° 64' N, longitude 6° 58' W), in the province of Salamanca (Spain). The study area covered 40.5 hectares (Fig. 1B) dominated by shrublands composed of *Cytisus multiflorus* (L'Hér.) Sweet on poor and dry soils, and pastures with livestock activity (only cattle) and groves of *Pyrus bourgaeana* Decne. (Fig. 1C) on rich soils with temporary waterlogging. It is under these *P. bourgaeana* groves where *O. × vasconica* lives, and its growth and flowering coincide with the temporary waterlogging period between March and April. In addition, other orchid species such as *Anacamptis morio* (L.) R. M. Bateman, Pridgeon & M. W. Chase and *Serapias lingua* L. were also found scattered throughout the sampling area.

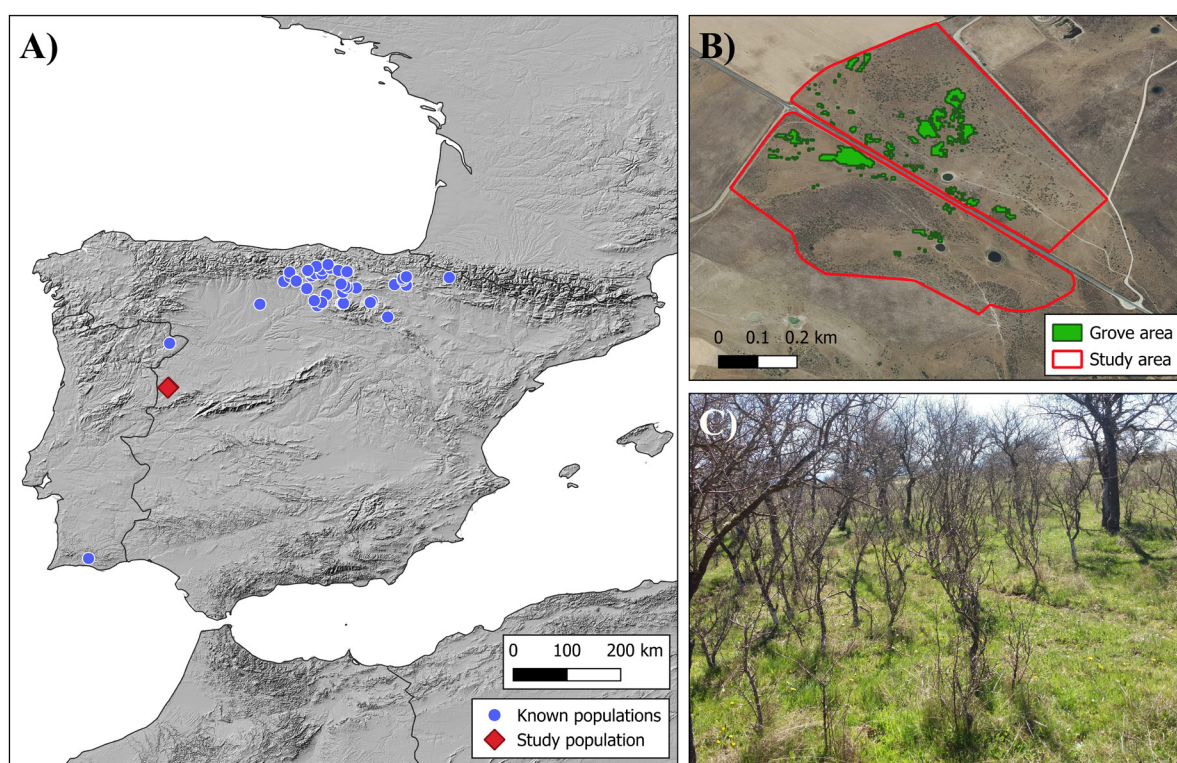


Figure 1. (A), map of the known chorology of *Ophrys × vasconica* (O. Danesch & E. Danesch) P. Delforge in the Iberian Peninsula, and the studied population in Saelices el Chico, Salamanca (red rhombus); (B), detail of the study area of 40.5 hectares (red area) and distribution of *Pyrus bourgaeana* Decne. groves (green area) where the studied population of *O. × vasconica* lives; (C), and general appearance of *P. bourgaeana* groves.

Data collection

Data on ecology, floral biology, and pollination rates were collected in April of 2017 and 2018. The month of April was chosen based on reported flowering dates in previous collections or field studies, considered to be the most appropriate for finding the population in a well-developed flowering state.

First, the entire sampling area was explored (Fig. 1B), identifying the subareas where *O. × vasconica* was present. After the initial exploration, and to determine the facilitating role of *P. bourgaeana* as a nurse plant in the growth of *O. × vasconica*, data on the distances of each *O. × vasconica* individual ($N = 299$) from the nearest *P. bourgaeana* trunk (measured from the central part of the leaf rosette) were collected in 2017.

For the study of the relationship between inflorescence size (floral spike) and number of open flowers, the total number of open flowers was counted in both years (total open flowers: $N = 311$ for 2017; $N = 549$ for 2018), and the total floral spike size was measured for each individual (total individuals: $N = 231$ for 2017; $N = 282$ for 2018). Each floral spike was measured from the base to the apex of the last flower. Additionally, to evaluate the pollination rates in the population, data on the presence or absence of pollinia (expressed as 0–1, respectively) were recorded for those open flowers. Individuals with the absence of pollinia in at least one of their flowers were considered to exhibit male pollination success (Parra-Tabla & Vargas, 2007).

Additionally, in May of 2017, data on fruiting was collected. The herbaceous grass vegetation inside the groves was very dense due to the lack of herbivory. For that reason, it was impossible to collect data on fruiting in all individuals of the population. A random sample of 60 out of 299 individuals was selected for data collection, recording the number of individuals with the presence of at least one developed fruit.

Data analysis

All analyses and graphics were performed using the R programming environment v4.2.3 (R Core Team, 2024). The distribution map and study area were made using QGIS software v3.34 (QGIS Development Team, 2024).

To observe the nurse effect of *P. bourgaeana* groves, the frequency data of distances to the nearest tree were represented in a density histogram.

To study the effect of explanatory variables on the variable of ‘floral spike size’, two linear ANOVA models (LM) were fitted: i) one to test the effect of distance to the nearest tree trunk; ii) and the second one to study differences in floral spike size between the two study years. Both models were examined for normality and homoscedasticity assumptions using Shapiro-Wilk and Levene tests, respectively. In both cases, the lack of normality and homoscedasticity was corrected by applying a logarithmic transformation to the response variable. In the second model, a sequential sum of squares Type III was used due to the different sample sizes between both years (Quinn & Keough, 2002).

On the other hand, Generalized Linear Models (GLMs) were performed for the response variables: ‘number of open flowers’ and ‘male pollination success’. An ANOVA GLM with Poisson error distribution was fitted to study the effect of the distance to the nearest tree trunk on the number of open flowers. Two full ANCOVA GLMs were fitted to study the effect of floral spike size (covariate) on the number of open flowers and on the probability of pollination. Both models included the factor year and the effect of the interaction between covariate and factor. After developing the complete models, interaction effects were removed as they were not significant ($p \geq 0.05$), and the models were re-adjusted, as recommended by Steinberg & Colla (2000). We used a Poisson error distribution for count-type data, and a binomial error distribution for presence-absence of pollinia (expressed as 0–1, respectively). Sequential sum of squares Type III (Wald Chi-square tests) was used for both ANCOVAs. The presence of overdispersion was tested as a measure of checking the model assumptions (Quinn & Keough, 2002), using the Goodness-of-Fit test from the aods3 package (Lesnoff & Lancelot, 2013).

RESULTS

The population size exhibited interannual variation. Specifically, there were 299 individuals recorded in 2017, whereas in 2018, the count only revealed 286 individuals.

It was observed that substrate accumulation around the trunks of *P. bourgaeana* resulted in the substrate not becoming waterlogged (Fig. 2A). It was within this substrate accumulation where *O. × vasconica* was found growing (Fig. 2B), remaining isolated from excessive water. As depicted in Fig. 3A, most individuals

grew within this substrate accumulation, with 81.27% of individuals ($N = 243$) growing at a distance less than 30 cm from the nearest trunk, and with a mean distance to the nearest tree of 21.87 ± 1.05 cm (mean $\pm$ SE; $N = 298$). The presence of only one individual growing outside the grove was observed, at 245 cm from the nearest tree.



Figure 2. (A), temporary waterlogging in the substrate of the *Pyrus bourgaeana* grove inhabited by *Ophrys × vasconica* in the studied population, where the abundant trampling by livestock can be observed on the waterlogged substrate; (B), and three *O. × vasconica* individuals growing isolated from the waterlogging and trampling by livestock, thanks to the nurse effect of *P. bourgaeana*.

Another noteworthy fact is that, out of the total number of individuals encountered in each sampling year (2017 and 2018, respectively), only five and four individuals exhibited damage caused by trampling and herbivory by livestock. This accounts for 1.68% and 1.40% of the total individuals in both years, respectively. The damaged individuals were found under small-sized trees, making them accessible to the animals.

The distance to the nearest tree trunk had a positive effect on floral spike size, with smaller distances associated with larger floral spikes (LM: $F = 6.58$, $P = 0.01$; Fig. 3B). On the other hand, the distance to the tree trunk had no effect on the number of open flowers per individual (GLM: $X^2 = 0.44$, $P = 0.51$; Fig. 3C).

Significant differences were found in the floral spike size between the two sampling years (LM: $F = 7.76$, $P < 0.01$; Fig. 4A), with smaller floral spikes observed in 2017 (mean $\pm$ SE = 8.89 ± 0.15 cm; $N = 293$) compared to 2018 (mean $\pm$ SE = 9.66 ± 0.18 cm; $N = 282$). Differences were also found in the number of open flowers (total open flowers: $N = 311$ for 2017; $N = 549$ for 2018) between both years (GLM: $X^2 = 23.94$, $P < 0.001$; Fig. 4B), with a lower number of open flowers observed in 2017 (mean $\pm$ SE = 1.34 ± 0.04 ; $N = 231$) compared to 2018 (mean $\pm$ SE = 1.94 ± 0.05 ; $N = 282$).

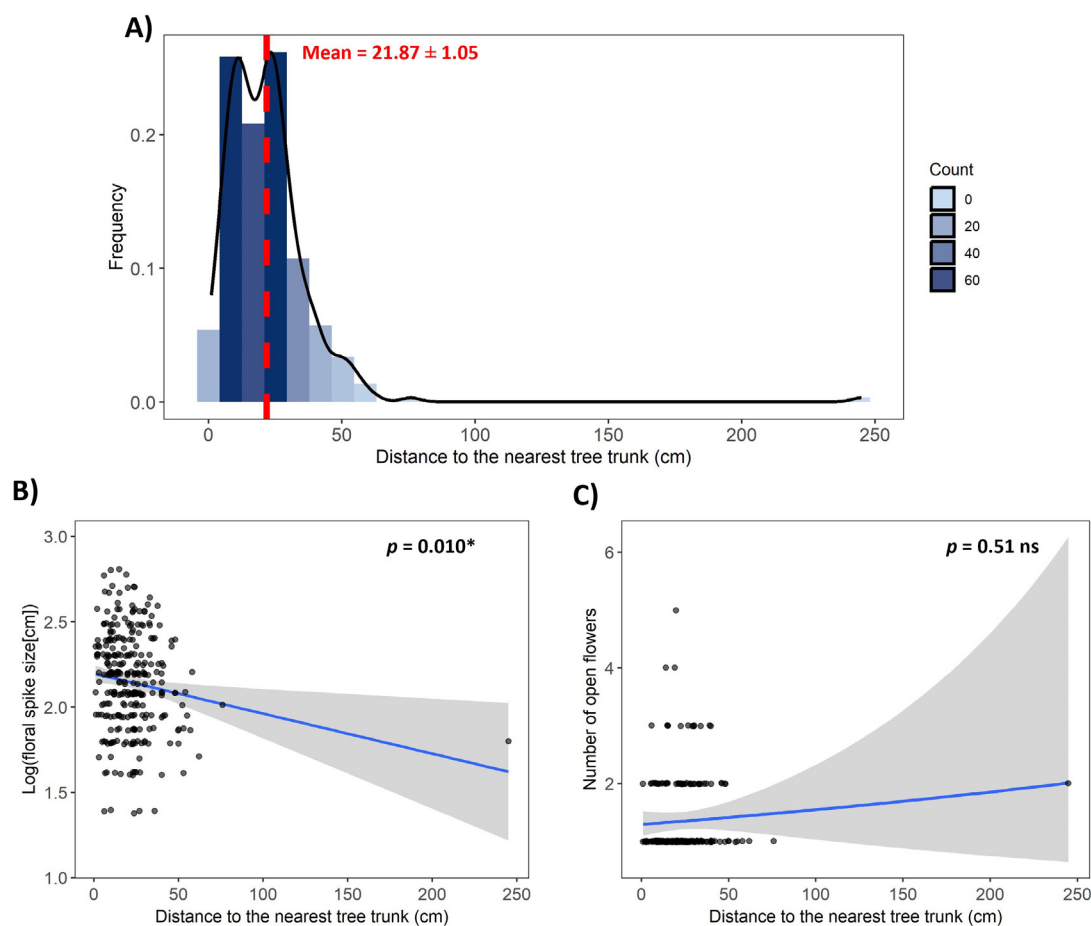


Figure 3. (A), histogram showing frequency and count of distances to the nearest nurse tree (cm). The red line represents the mean of the recorded distances; (B), linear relationship between the distance to the nearest tree trunk and the floral spike size (cm). The latter variable is logarithmically transformed to meet the assumption of normality; (C), relationship between the distance to the nearest tree trunk and the number of open flowers. Shaded areas indicate 95% confidence intervals.

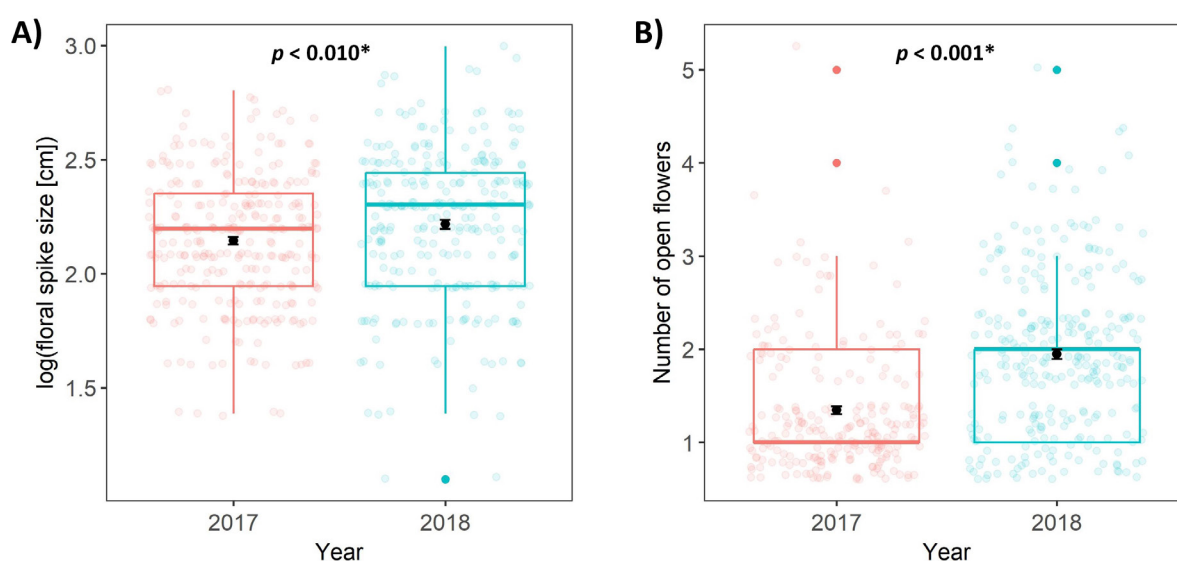


Figure 4. Differences in: (A), floral spike size (cm); (B), and number of open flowers per individual found in each year of sampling. The black dot and the thick line represent the mean (with the standard error) and the median, respectively.

There was a positive relationship between floral spike size and the number of open flowers, where individuals with larger floral spikes exhibited a higher number of open flowers (GLM: $X^2 = 17.62$, $P < 0.001$; Fig. 5A). The model did not report a significant effect of the interaction between covariate and factor (floral spike size*year: $X^2 = 1.97$, $P = 0.16$). Similarly, floral spike size had a positive effect on male pollination success, where the individuals with larger floral spikes were more likely to have their pollinia removed on at least one of their flowers (GLM: $X^2 = 64.00$, $P < 0.001$; Fig. 5B). Although there was no interaction between covariate and factor (floral spike size*year: $X^2 = 0.17$, $P = 0.67$), male pollination success varied significantly between the two years (GLM: $X^2 = 15.15$, $P < 0.001$), with a higher probability observed in 2018.

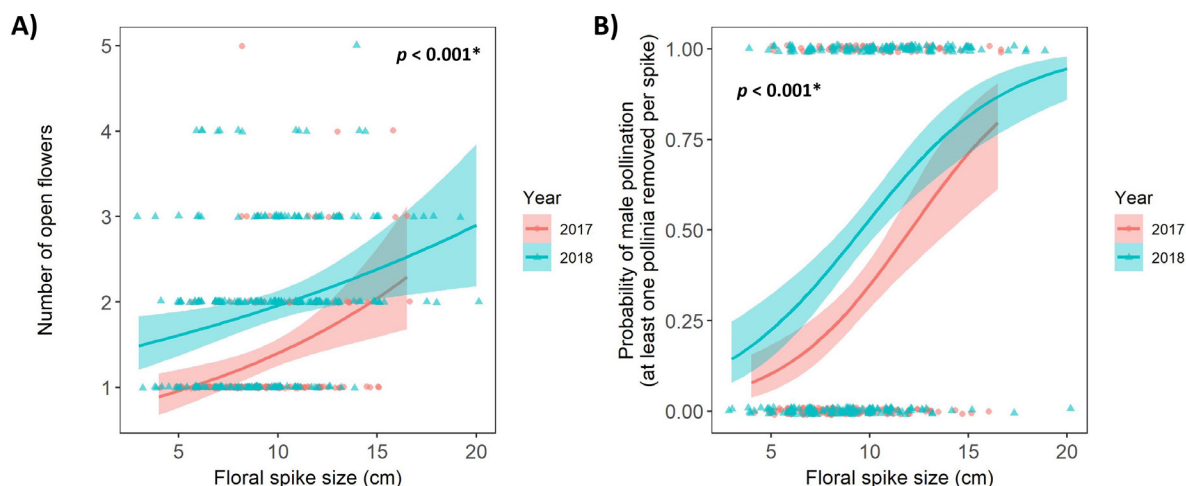


Figure 5. (A), relationship between floral spike size (cm) and the number of open flowers for the sampling years 2017 and 2018, according to a GLM with Poisson error distribution and Analysis of Deviance Type III (Wald Chi-square tests); (B), and relationship between floral spike size (cm) and male pollination success (at least one pollinia removed per floral spike) for the sampling years 2017 and 2018, according to a GLM with binomial error distribution and Analysis of Deviance Type III (Wald Chi-square tests). Shaded areas indicate 95% confidence intervals.

The male pollination success in individuals with open flowers was 25.51% in 2017 ($N = 75$ individuals with pollinia removed) and 50.53% in 2018 ($N = 143$). Meanwhile, out of the 60 individuals sampled in 2017, 58 showed developed fruits (female pollination success), representing 19.79% of the entire population ($N = 293$; the five individuals with inflorescence damage caused by herbivory were excluded from the total count).

DISCUSSION

The number of individuals in this disjunct population of *O. × vasconica* seems to be variable between years (298 and 286 individuals recorded in 2017 and 2018, respectively). This fact is not unusual in the demography of mediterranean orchids, where population size will depend on annual mortality and recruitment rates, as well as periods of dormancy, which vary between years based on the climatic conditions experienced in the previous year (Pfeifer *et al.*, 2006; Hutchings, 2010; Romano *et al.*, 2020). Nevertheless, a difference of only 13 individuals (approximately 4% of the total population size) should be interpreted with caution.

The present study revealed some previously unknown characteristics about *O. × vasconica*. It highlighted that the presence and long-term maintenance of the studied population are likely due to the positive interaction with the *P. bourgaeana* groves under which the orchid lives. The low number of damaged individuals demonstrates the nurse effect provided by the groves, protecting the orchid from herbivory and trampling by livestock (only cattle). Additionally, the groves may also be improving the edaphic and microclimatic conditions for the orchid. On one hand, the accumulation of substrate around the tree trunks isolates the orchid from excessive waterlogging of the substrate during the rainy period (spring),

which coincides with seed germination and tuber regrowth. In this case, the excess waterlogging would be caused by substrate compaction because of livestock trampling (Sharrow, 2007; Chyba *et al.*, 2014). And on the other hand, it may protect the orchid from excessive temperature and radiation, as well as retain substrate moisture during the summer, a period coinciding with the end of fruiting and seed dehiscence. This is important because in mediterranean climates, particularly during hot and dry summers, mortality could increase outside the groves, thus decreasing seed production. Likewise, the potential role of *P. bourgaeana* in promoting the presence of mycorrhizal fungi in the soil should not be overlooked, since these fungi are essential for orchid seed germination and local population establishment (Li *et al.*, 2021). However, this aspect has not been evaluated in the present study.

The initial hypothesis of ‘nurse groves’ is supported by the results obtained. More than 80% of the individuals grew at less than 30 cm from the tree trunks (Fig. 3A), developing larger floral spikes as the proximity to the nurse trees increased (Fig. 3B). These findings are the first time they have been reported for the genus *Ophrys* and are consistent with results reported for other terrestrial orchid species in mediterranean climates (such as *Bipinnula fimbriata* (Poepp.) I. M. Johnst., *Chloraea galeata* Lindl. and *C. bletioides* Lindl.), where the presence of nurse plants also improved edaphic and microclimatic conditions, enhancing growth while reducing herbivory damage (Badelomar *et al.*, 2019; Atala *et al.*, 2020). Furthermore, other authors have also reported that an excess of nitrates in the substrate inhibits the germination of terrestrial orchid seeds (Figura *et al.*, 2020, 2021). Hence, a potential increase in nitrates in the soil from livestock excrement could contribute as an explanation for the absence of *O. × vasconica* outside the groves and as a result, growth on the substrate accumulation could be isolating both established individuals and seeds from nitrates dissolved by runoff. Considering that the suitability of germination sites in orchids sometimes differs by a few centimetres (Phillips *et al.*, 2011), the groves could also play an important nurse role by facilitating or allowing germination. However, empirical evidence on germination is needed to further test this hypothesis in *O. × vasconica*.

Both floral spike size and number of open flowers differed between years. We attributed this to interannual variations in abiotic conditions such as temperature or precipitation (Janeckova & Kindlmann, 2002). However, it is worth noting that both floral spike size and the number of open flowers found are smaller than those reported for species of the genus *Ophrys* such as: *O. baelarica* P. Delforge, *O. bilunulata* Risso, *O. exaltata* Ten., *O. fuciflora* (F. W. Schmidt) Moench, *O. fusca* Ten., *O. insectifera* Gueldenst., *O. lutea* Cav., *O. sphegodes* Mill. and *O. tenthredinifera* Willd. (Hutchings, 1987; Dorland & Willems, 2002; Vandewoestijne *et al.*, 2009; Scopece *et al.*, 2015; Borràs-Riera, 2018). On the contrary, they are similar to those reported for *O. bertolonii* Moretti (Scopece *et al.*, 2015).

The positive relationship found between floral spike size and reproductive success is consistent with findings reported for other orchids and *Ophrys* species, and it can be explained by two possible factors. First, reproductive success in plants like food deceptive orchids increases with the size of the inflorescence. This is because having larger inflorescences or longer floral spikes enhances visibility for pollinating (Johnson & Nilsson, 1999; Maad & Alexandersson, 2004; Dickson & Petit, 2006), facilitating their location among other plants. However, in sexually deceiving orchids like the genus *Ophrys*, the increase in visibility for male pollinators may be more due to olfactory cues than visual cues (Vandewoestijne *et al.*, 2009). This is evidenced by studies on species such as *O. sphegodes*, which have shown that scent is the most important factor for effective pollination to occur (Schiestl *et al.*, 2000), indicating that while visual cues may also play an important role at short distances, the emission of large quantities of female pheromones is more crucial for long-distance attraction. The second factor, as authors such as Valdivia & Niemeyer (2006) have reported, might be that the quantity of pheromones depends on the number of flowers. Therefore, if the number of flowers increases with the size of the inflorescence or floral spike, it is logical that pheromone emission will increase with the size. Consequently, plants with larger inflorescences or floral spikes will have higher reproductive success. The results found in the studied population of *O. × vasconica* support this hypothesis, as they report both a greater number of flowers and a higher probability of pollination in plants with a larger floral spike size (Fig. 5).

On the other hand, it should be noted that measuring pollination or the probability of pollination based on the absence of pollinia provides only a partial assessment of reproductive success (male reproductive success), since it does not account for whether the pollen actually reaches the stigmas and fertilization occurs (female reproductive success), nor does it consider the potential accidental loss of pollinia. However,

given the large number of individuals sampled, using the absence of pollinia as a measure of pollination is thought to compensate for any accidental pollinia removal. In addition, in this study, male reproductive success was assessed based on the removal of pollinia from at least one flower per floral spike, rather than recording the presence or absence of pollinia in all flowers of each individual. This approach is not considered a methodological limitation, as previous studies have shown that, in rewardless deceptive orchids, pollinators typically leave quickly, resulting in only a few flowers being visited per floral spike, even when inflorescences contain many flowers (Rodríguez-Otero *et al.*, 2022; Traxmandlová *et al.*, 2025).

Finally, the fact that 58 out of 60 sampled individuals (19.79% of total population) had developed fruits indicates a high female pollination success. We believe this data is not particularly significant as the sampling was not conducted on the entire population due to the dense growth of grass vegetation (developed within the *P. bourgaeana* groves), making it impossible to collect data on all individuals of *O. × vasconica*. However, the presence of at least 19.79% fruit development is consistent with fruiting data reported for other populations of the genus *Ophrys* (Wolff, 1950; Borràs-Riera, 2018) and other mediterranean orchid genera (Schatz, 2006; Capó *et al.*, 2021).

In conclusion, the results show that *P. bourgaeana* groves, where the orchid *O. × vasconica* lives, act as nurse plants, a condition not previously noted in other disjunct populations of this species or in other species of this genus. This means that the orchids are more protected from detrimental temporary waterlogging during the flowering season, as well as from herbivory, both caused by livestock (only cattle). The results also indicate that the size of the floral spike decreases with distance from the nurse tree trunks. This translates into increased male reproductive fitness the closer individuals are to the nurse trees, as individuals with longer floral spikes exhibited higher male pollination success.

Overall, our study highlights the importance of the presence of these groves in terms of the conservation of the studied population. Practices such as logging or burning of the groves to increase grassland area can endanger the population, reducing both population size and reproductive success. In addition, since the presence of cattle causes compaction of the grasslands and its subsequent waterlogging, the delimitation and maintenance of *P. bourgaeana* woodland areas, inaccessible to livestock (temporarily fenced, for example), may promote the increase the populations of this orchid.

Finally, it would be interesting to conduct studies of this kind in other populations of this orchid species (whether disjunct or not), to verify if the association with nurse plants is also present.

Supplementary information ↑

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Supplementary material

Not applicable.

Data availability

Not applicable.

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Authorship contribution statement

Jaime García-Gila: all applicable roles for this manuscript, according to the CRediT taxonomy, have been performed by the undersigned author.

Competing interests

Not applicable.

Statement on the use of Artificial Intelligence

Not applicable.

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