www.biodicon.com Biological Diversity and Conservation

ISSN 1308-8084 Online ISSN 1308-5301 Print

Research article
DOI: 10.46309/biodicon.2024.1478456
18/1 (2025) 20-30

Floral biology, pollination and reproductive success of *Campanula tomentosa* Lam. in west Anatolia

Ümit SUBAŞI [1], Aykut GÜVENSEN [*1]

ORCID: 0000-0002-8003-6227**;** 0000-0002-6384-3668
Ege University Science Faculty, Biology Department, Bornova, 35100 Izmir, Türkiye

Abstract

Campanula tomentosa, an endemic species, is distributed only in the Western Anatolia Region of Türkiye. Research on the reproduction and conservation biology (pollination biology and reproductive success) of the plant was carried out for the first time. Pollen and stigma viability (floral biology), pollination biology, flower visitation, pollinator identification and reproductive success were investigated. The results of the pollen viability test in which 1% TTC (1,2,3-triphenyl tetrazolium chloride) was used indicated that 100% of the pollen grains were viable at the loading stage. In Peroxidase Test Papers and DAB stigma viability tests, the stigmas were completely curled and they were viable during the pollen presentation phase. The main pollination in the plant was realized by *Xylocopa valga* (Gerstacker, 1872) and *Evylaeus setulellus* (Strand, 1909). In *C. tomentosa*, fruit set and seed setting rates were 93.42% and 73.12%, respectively. On the other hand, it was revealed that the total area where it was distributed was 28.7 km^2 and that it was classified as endangered.

Keywords: *Campanula tomentosa*, reproductive success, pollen viability, Türkiye

---------- * ----------

Batı Anadolu'da yayılış gösteren *Campanula tomentosa* Lam'ın çiçek biyolojisi, tozlaşması ve üreme başarısı

Özet

Endemik bir tür olan *Campanula tomentosa*, Türkiye'nin sadece Batı Anadolu Bölgesi'nde yayılış göstermektedir. Bitkinin üreme ve korunma biyolojisi (tozlaşma biyolojisi ve üreme başarısı) üzerine araştırmalar ilk kez tarafımızdan gerçekleştirilmiştir. Bu kapsamda polen ve stigma canlılığı (çiçek biyolojisi), tozlaşma biyolojisi, çiçek ziyareti, tozlayıcıların tanımlanması ve üreme başarısı araştırılmıştır. %1 TTC'nin (1,2,3-trifenil tetrazolyum klorür) kullanıldığı polen canlılık testi sonuçları, yükleme aşamasında polen tanelerinin %100'ünün canlı olduğunu göstermiştir. Peroksidaz Test Kağıtlarında ve DAB stigma canlılık testlerinde stigmalar tamamen kıvrılmış ve polen sunum aşamasında canlı kalmıştır. Bitkide esas tozlaşma Xylocopa valga (Gerstacker, 1872) ve Evylaeus setulellus (Strand, 1909) tarafından gerçekleştirilmiştir. *C. tomentosa*'da meyve tutumu ve tohum tutumu oranları sırasıyla %93,42 ve %73,12'dir. Ayrıca, yayılış gösterdiği toplam alanın 28,7 km^2 ve neslinin tükenme kategorisi sınıfında olduğu tespit edilmiştir.

Anahtar kelimeler: *Campanula tomentosa*, üreme başarısı, polen canlılığı, Türkiye

[*] *Corresponding author*: Tel.: +902323112434; Fax.: +902323881036; E-mail: aykut.guvensen@ege.edu.tr
 Received: 04.05.2024; Published: 15.04.2025 BioDiCon. 1155-040524

1. Introduction

According to the results of the global screening conducted by the International Union for Conservation of Nature (IUCN) in 2020, of the 240,000 known plant species to date, about 15% are endangered. Of these endangered species, more than 90% are endemic. These plant species in the world are pollinated by approximately 300,000 animal species [1]. On the other hand, it is estimated that 25,000 to 30,000 plant species are pollinated only by bee species. Environmental variables such as elevation, ambient temperature, wind speed, relative humidity and light intensity, and demographic characteristics such as population size of a plant species are closely related to the behavior and number of pollinators [2].

The genus *Campanula L.* is represented by approximately 420 species in subtropical and temperate regions and approximately 150 species in the Mediterranean Region [3]. In many countries, studies have been carried out on the genetic diversity and polymorphism, pollination and reproductive system, population biology and conservation biology of the *Campanula* taxa. For instance, it was investigated the genetic diversity of populations of *Campanula* taxa [4]. Conservation genetics were studied in the *Campanula rotundifolia* L. population [5]. In another study, it was carried out studies aimed at identifying the flowering phenology of *Campanula bononiensis* L., and determining flowering abundance, population density and pollinators observed on the same species [6].

In Türkiye, the family Campanulaceae is represented with the following 6 genera: *Campanula, Symphayndra, Asyneuma, Michauxia, Legousia* and *Jasione*. Of these genera, Campanula has 6 sub-genera: *Campanula, Megalocalyx, Sicyodon, Roucela, Brachycodonia and Rapunculus* [7]. In these sub-genera, there are 139 taxa belonging to 127 species and the endemism rate is approximately 52% [8]. Of these sub-genera, the *Quinqueloculares* section, one of the 13 sections of *Campanula* subgenus which has the highest number of species, is represented by 9 species and 6 of these species are endemic to Anatolia. Our review of the studies conducted on this species in Türkiye demonstrated that investigated the anatomy of some *Campanula* taxa, investigated the morphology of seeds of *Campanula* species (sect of *Quinqueloculares*), and some soil parameters were revealed [9]. In other studies, endemic *Campanula tomentosa* Lam. and *C. vardariana* Bocquet species, seed germination studies were carried out [10], and conservation biology of the species *Campanula teucrioides* Boiss (Campanulaceae) was investigated [11]. Türkiye is rich in endemic species, and the ratio of the endemism to the total flora is about 34.4%. In recent years, in Türkiye, emphasis has been placed not only on autoecologic studies on the protection of endangered plants but also on studies aiming to reveal life-cycles of these plants [12].

In the present study, reproduction and conservation biology of endemic *C. tomentosa* which has a limited distribution in the Western Anatolia region of Türkiye was studied for the first time. Within this context, pollen and stigma viability (floral biology), pollination biology, flower visitation and pollinator identification and reproductive success were investigated. In addition, the number of individuals in the plant population and the areas they are distributed in were calculated, and its place in the IUCN Red List Categories was updated [13].

1.1. Study Sites and Species Description

The study was conducted on 5 populations of *C. tomentosa* distributed in Meryem Ana (Izmir); National Park-South of Doğanbey, National Park- North of Doğanbey, Çamlık-Ortaklar, and Akçakonak-Priene (Aydin) located in the Aegean region of Türkiye between May 2012 and September 2014 (37º55 '- 37º39'N and 27º27 '- 27º09' E) (Figure1). The average annual temperature in the region is 16.7°C, and the annual average rainfall is 659.4 mm. The geologic structure of the region is reported to have marble, schist and calcschist character.

C. tomentosa has a woody base and branched stem is 30-70 cm in height, and is covered with flat, dense and long tomentose hair. Stem leaves are oval-, oval-triangular- or lyrat-shaped, sessile or petiolate, 1.5-7.5 x 0.7-2.7 cm, with crenate-serrate edges. The flowers are violet-blue. The corolla is 26-53 x 18-45 mm in size, has a bell-like or wide funnel shape, and its outer part is hairy. Corolla tube is 18-42 x 12-37 mm in size, stigma and ovary each has 5 loculi, and fruits have a porocidal capsule. While the pistil is 13-37 x 2.5-3.5 mm in size, the stamen is 8-18 x 0.9-1.1 mm. The capsule has dense tomentose hairs, and the seeds are 0.4-0.5 x 0.25-0.35 mm in size, oval-shaped, and mostly light brown. Flowering season is from May to June. It grows on walls and bottom of rocks and calcareous rocks at elevations ranging between 1 and 100 m. It is endemic to Eastern Mediterranean region.

2. Materials and methods

2.1. Floral Biology

To determine its floral morphology, 30 flowers and flower buds were brought to the laboratory in FAA (acetic acid 5%, formaldehyde 5% and ethanol 90%). In the laboratory, the diameter and length of the corolla tube, height and width of the calix, flower size and pistil lengths in mature flowers were measured using a digital caliper and a digital microscope (Dino-Lite AM313). On the other hand, in 15 individuals, stigma lobes, anther and style lengths, stigma diameters, stigma-anther distance in mature flowers (with pollen presentation) and flower buds (without pollen

presentation) were calculated. Voucher specimens of *C. tomentosa* were deposited in the EGE Herbarium at the Department of Botany, Ege Universty (EGE – 41688, EGE – 41689; EGE –41690; EGE – 41691).

Nectar volumes were measured between 09:00 and 10:00 a.m., 12:00 and 1:00 p.m. and 4:00 and 5:00 p.m. using 0-5µ micropipettes in 30 flowers from the randomly selected individuals in the distribution areas of the species. Sugar concentration in these samples was determined using a hand refractometer, 0-50% Brix (Bellingham and Stanley model 45-81, Tumbrige Wells, UK). The total sugar content was calculated using the exponential regression formula [14].

Pollen viability was tested at the pollen presentation and pollen loading stages. To carry out the test, 1% TTC (1,2,3-triphenyl tetrazolium chloride) was applied on 20 flowers and flower buds. After this application, to examine the slides, a light microscope (Leica: 10x18 ocular; 4x/0.10 objective) was used. The numbers of viable (%) and nonviable (%) pollen grains were counted from ten randomly chosen fields of view at 10x. After the pollen preparations were first stained and then incubated at 30°C, they were examined under the light microscope at 160-400x magnification. To calculate the viability percentages of pollen taken from anthers of flower buds and flowers, 100 pollen grains were used. The procedure was repeated 5 times.

To determine stigma viability, Peroxidase Test Papers (Perex Tesmo KO, Macherey-Nagel) and the DAB test (Sigma FastTM 3.30-diaminobenzidine tablets; Sigma D-4168) were used [15]. These applications were tested for different floral phases.

2.2. Flower Visitation and Pollinator Identification

These observations were performed in randomly selected 15 individuals for 3 days between 09:00 a.m. and 10:00 a.m., between 12:00 p.m. and 1:00 p.m. and between 4:00 p.m. and 5:00 p.m. when the weather conditions were appropriate (sunny and slightly windy). Pollinators observed were caught and brought to the laboratory in alcohol (70%). Identification of the pollinators was performed at Hacettepe University Faculty of Science, Department of Biology, Department of Zoology. In addition, the collections belonging to these pollinators were recorded with ZDEU24 / 2013-ZDEU46 / 2013 inventory numbers in the museum, part of the Department of Zoology, Department of Biology, Ege University Faculty of Science.

2.3. Reproductive success

To demonstrate the reproduction success of the species, such parameters as (1) Fruit Set Rate (FSR), (2) Seed Setting Rate (SSR) were used.

2.4. The Effect of Cross Pollination on Seed Production

To determine the rates of fruit set, 100 flowers (two flowers per individual) were randomly selected from the *C. tomentosa* populations and labeled, and then the average number of flowers per plant (n = 50) and the average number of fruits per plant (n = 50) were calculated.

The Fruit Set Rate was calculated using the following equation:

Fruit Set Rate (%) = the average number of fruits per plant x 100 ÷ the average number of flowers per plant

To determine the Seed Setting Rate, firstly, the average number of seeds per fruit and the average number of ovules per flower were calculated. While calculating the average number of seeds per fruit, the number of the seeds from 100 mature fruits of 50 individuals (two fruits per individual) was determined. When the average number of ovules per flower was calculated, the ovaria of 200 flowers (not in the seed-bearing stage) taken from 20 individuals (10 ovaria per individual) were opened, and then the number of ovules was counted using a light microscope.
Seed Setting Rate (%) was calculated using the following equation:

Seed Setting Rate (%) = the average number of seeds per fruit x 100 ÷ the average number of ovules per flower

2.5. The Effect Self - Pollination on Seed Production

In order to determine the pollination type of the species, 100 flowers from 50 individuals were taken using pollination bags to repel possible pollinators. After about 1 month, flowers that were packed were brought to the laboratory, and fruit and seed development was observed.

2.6. Determination of the Number of Individuals and the Size of the Distribution Areas

The average number of individuals in 5 populations of *C. tomentosa* was calculated as 50 by taking sample areas of 10m x 10m (100 m^2) between May 2013 and July 2013. The Average Number of Individuals calculated was

adapted to all populations and the number of potential individuals in the distribution area was calculated. All of the aforementioned research was carried out in population in the National Park- South of Doğanbey. When calculating the size of the distribution area for each population, first the spatial data were recorded using the GIS (Geographic Information System), including the boundary distribution areas in the areas where the plant was distributed. Then the recorded spatial data were transferred to the "Google Earth Pro" program, and km. extension polygons were created. Finally, numerical maps were created by transferring the generated polygons to "ArcMap ver.10.0" program and field sizes were calculated. Thus, the total distribution area of the plant and the number of potential individuals were calculated. In the light of these data, *C. tomentosa's* place in the IUCN Red List Categories (2001) Ver. 3.1 was updated.

3. Results

3.1. Floral Biology

The measurements made on 30 flowers belonging to *C. tomentosa* demonstrated the following average values: corolla diameter: 34.52 mm, corolla length: 34.76 mm, calyx length: 20.47 mm, calyx width: 8.20 mm, and pistil length: 33.67 mm (Table 1). As a result of our floral morphology study, the phase at which the "Secondary Pollen Presentation" took place was determined. Our observations demonstrated that pollen loading (adhesion of pollens to styles by anthers) occurred when the stigma lobes were closed prior to anthesis, that stigma lobes gradually curled backward after anthesis (secondary pollen presentation), and that stigmas were completely curled in the fully opened flowers. At the end of the pollen presentation, mean floral measurements of *C. tomentosa* flowers showed that the stigma lobe length was 5.45 mm, stigma diameter was 5.61 mm, stigma-anther distance was 3.35 mm, anther length was 13.49 mm, and style length was 24.78 mm.

Table 1. Floral morphological measurements of *C. tomentosa*

Floral features		*C. tomentosa*	
		Min-Mak (mm)	Average (mm)
Corolla diameter		23,40-43,34	34,52±5,93
Corolla length		29,25-39,57	34,76±2,81
Calix	Length	15,60-28,22	20,47±3,09
	Width	5,00-10,78	8,20±1,31
Pistil		29,01-39,64	33,67±2,38
Stigma diameter		2,78-8,6	5,61±1,56

In *C. tomentosa*, the average values of these floral measurements in the flowers at the pollen loading phase were 4.12 mm; 2.85 mm, 2.36 mm; 12.66 mm and 16.52 mm, respectively (Table 2). During the day, average sugar values and sugar weight per volume for the flowers of *C. tomentosa* were measured. The highest value of sugar was measured in the evening (Table 3).

Table 2. Morphological measurements of pistil and stamen in flowers at the pollen loading and presentation stages of *C. tomentosa*.

Stage	The number of the individuals (n)	Stigma lobe length (mm)	Stigma diameter (mm)	Stigma-anther distance (mm)	Anther length (mm)	Style length (mm)
Pollen loading	15	4,12±0,69	2,85±0,90	2,36±2,14	12,66±0,77	16,52±1,65
Pollen presentation	15	5,45±1,24	5,61±1,56	3,35±1,33	13,49±1,03	24,78±3,33

Table 3. Nectar values in *C. tomentosa* flowers measured during the day.

Times	The Number of Flowers Used		Sugar Concentration (%)	Nectar in micro pipette (cm)	Net Volume V (µl)	Sucrose (mg /µL)
09:00 a.m 10:00 a.m	30	Ort.±SH	34,93±10,67	0,76±0,65	1,26±1,08	0,40±0,14
		min-mak	19,00-52,00	0,15-2,3	0,25-3,83	0,20-0,64
12:00 p.m 1:00 p.m	30	Ort.±SH	41,76±13,89	0,74±0,51	1,24±0,85	0,50±0,18
		min-mak	10,00-60,00	0,20-2,5	0,33-4,16	0,10-0,77
4:00 p.m 5:00 p.m	30	Ort.±SH	45,24±7,18	0,99±0,65	1,64±1,08	0,54±0,10
		min-mak	32,00-54,00	0,30-2,8	0,50-4,66	0,36-0,67

For pollen viability, 1%TTC (1,2,3-triphenyl tetrazolium chloride) test applied to 20 *C. tomentosa* flowers. Of the 538 pollen grains counted during the pollen presentation phase (the stage when the pollen is in the style, 382 were stained, and pollen viability was calculated as 71% on average. On the other hand, during the pollen loading stage (the stage when the pollen is not in the style), pollen grains in the anthers belonging to 20 flower buds from ten fields of view at 10× (4cm^2) were counted and pollen viability was determined as 100%. According to the results of peroxidase test papers and DAB stigma viability tests on 20 flowers and flower buds at different floral phases belonging to *C. tomentosa*, stigmas at the pollen loading phase and stigmas in the presentation phase but not curled were found to be non-viable (no staining, negative). On the other hand, it was determined that the stigmas which were completely curled in the pollen presentation phase were viable (positive staining) (Table 4).

Table 4. Pollen / stigma viability of flowers in different floral phases.

	Pollen loading stage	Pollen presentation stage
Pollen viability (%)	100	71,00±6,45
Stigma viability	No staining, Negative	Staining, Positive
Stigma lobe length (mm)	4,12±0,69	5,45±1,24
Style length (mm)	16,52±1,65	24,78±3,33

3.2. Flower Visitation and Pollinator Identification

In our observations, *C. tomentosa* was visited by only 2 pollinators between 11:00 a.m. and 3:00 p.m.: *Xylocopa valga* (Gerstacker, 1872) from the Apidae family and *Evylaeus setulellus* (Strand, 1909) from the Halictidae family. In addition, the following passive pollinators were observed: members of the Curculionidae, Hemiptera and Cantharidae (Table 5). While *Xylocopa valga* did not visit the plant between 09:00 a.m. and 10:00 a.m., between 3:00 a.m. and 4:00 p.m. and between 4:00 p.m. and 5:00 p.m., *Evylaeus setulellus* did not visit the plant between 09:00 a.m. and 10:00 a.m., between 10:00 a.m. and 11:00 a.m., between 3:00 a.m. and 4:00 p.m. and between 4:00 p.m. and 5:00

p.m. (Figure1). *X. valga* visited the plant most during the day between 10:00 a.m. and 11: 00 a.m. (145 times) and the mean duration of visits was 17.5 ± 4.68 seconds. *E. setulellus* visited the plant most during the day between 11:00 a.m. and 12:00 p.m. (12 times) and between 1:00 p.m. and 2:00 p.m. (12 times), and the mean duration of visits was 30.58 ± 8.1 seconds. The temperatures during the day varied from 19.04ºC (09:00 a.m.) to 27.02ºC (17:00 p.m.) (Figs. 2, 3).

Table 5. Flower visitors of *Campanula tomentosa*

Flower Visitors	Category	Resource Used*
Xylocopa valga (Gerstacker, 1872)	L	P/N
Evylaeus setulellus (Strand, 1909)	L	P/N
Curculionidae spp.	O	P
Hemiptera spp.	O	P
Cantharidae spp.	O	P

Abbreviations: L = legitimate pollinator; O = occasional pollinator, *Floral resources used by the visitors/pollinators: P: pollen; N: nectar.

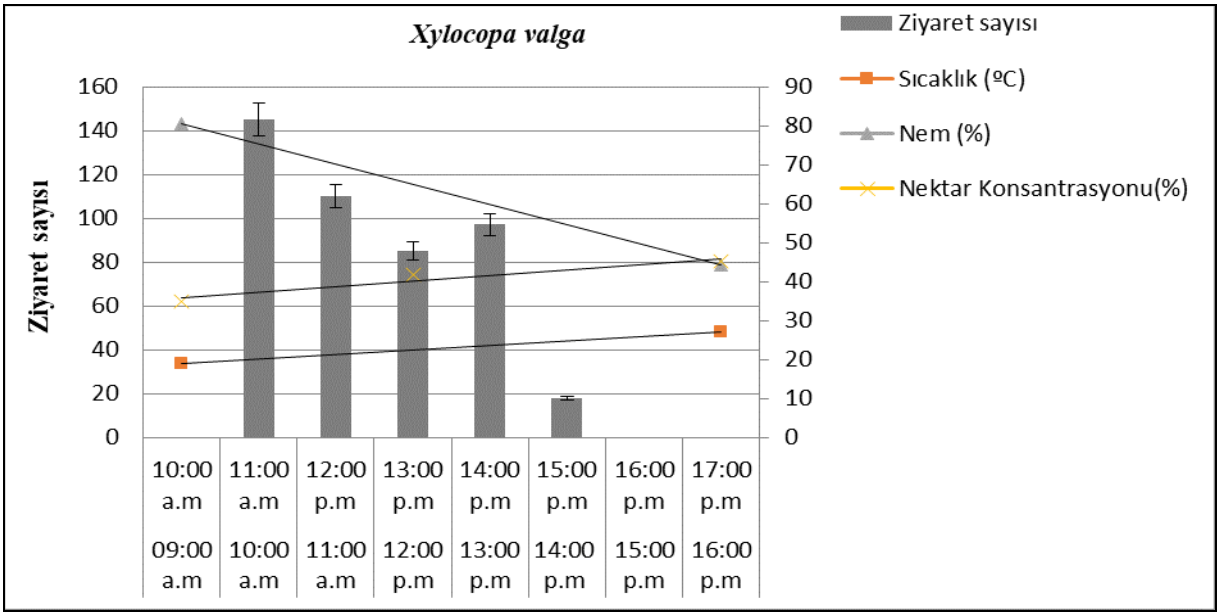

Figure 1. The number of flower visits by *Xylocopa valga* (Gerstacker, 1872), and temperature and humidity values

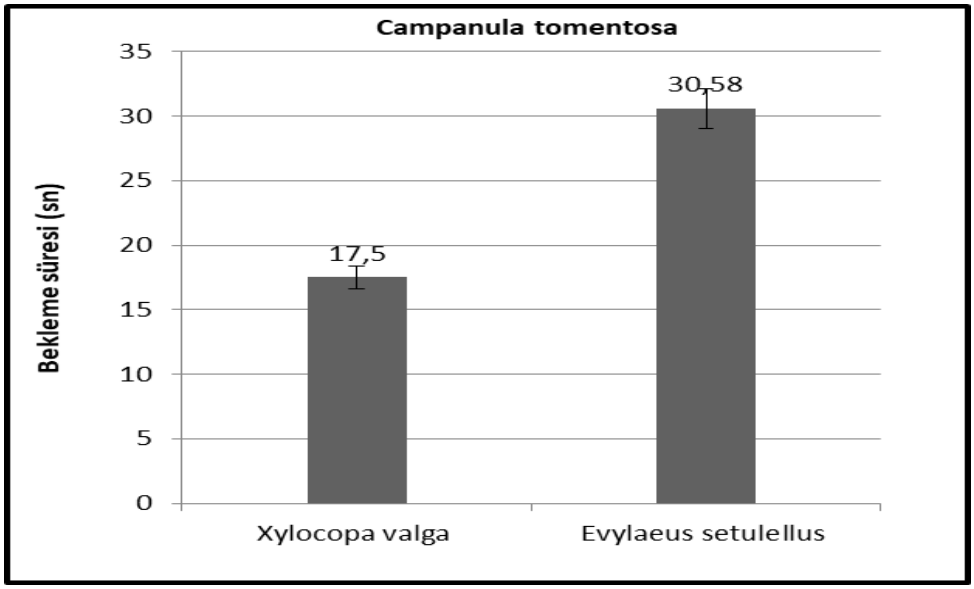

Figure 2. Mean duration of visits by *C. tomentosa* pollinators

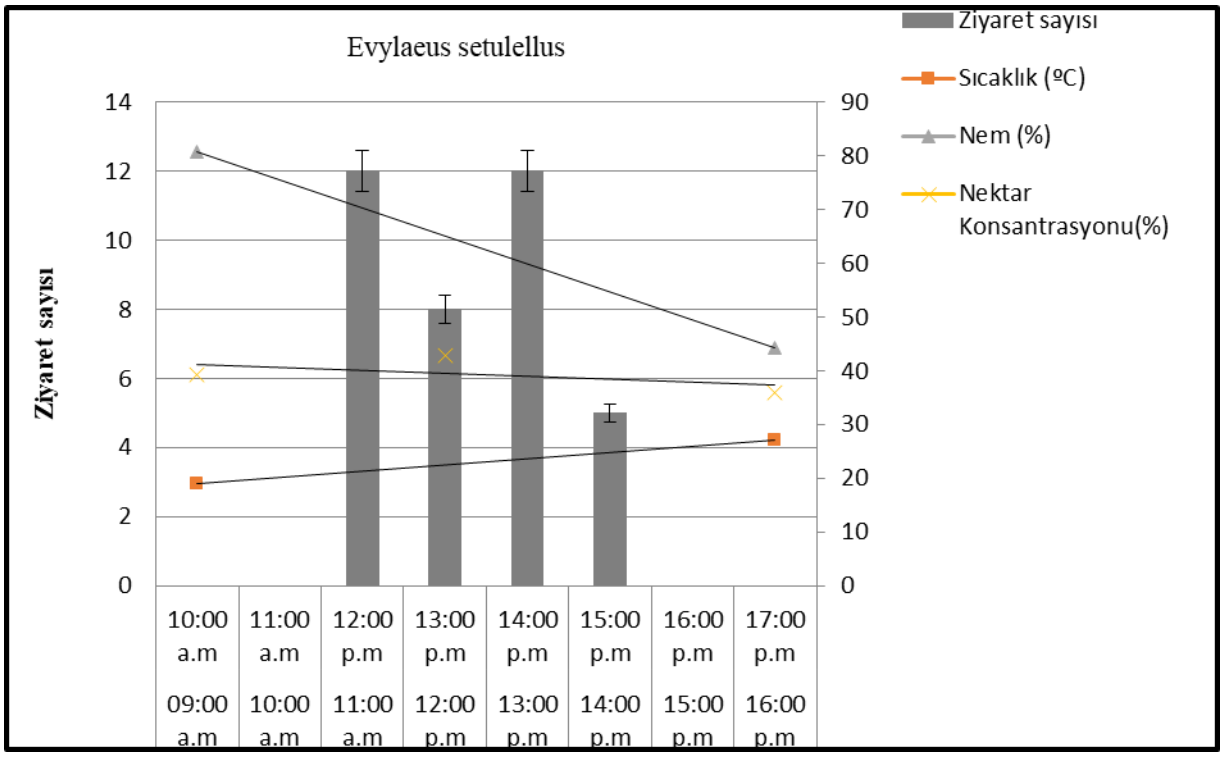

Figure 3. The number of flower visits by *Evylaeus setulellus* (Strand, 1909), and temperature and humidity values

3.3. Reproductive success

At the end of the trials on the effects of cross pollination on seed production in individuals exposed to pollinator visits under natural conditions, the average number of flowers per *C. tomentosa* plant was calculated as 110.64. The fruit counts of *C. tomentosa* demonstrated that the average number of seeds per fruit was 726.12 and the average number of ovules per flower was 992.94. At the end of the seed counts of these fruits, seed setting rate of the ovules was 73.12% (Table 6).

Table 6. Comparison of reproductive success parameters of *C. tomentosa*

Reproductive success parameters	The average number of flowers per plant	The average number of fruits per pant	Fruit set rate (%)	The average number of seeds per fruit	The average number of ovules per flower	Seed setting rate (%)
n	50	50	93,42	50	20	73,12
Min.-Mak.	22-207	11-202		510-1152	500-1198	
Ortalama	110,64±49,38	103,36±48,11		726,12±118,02	992,94±205,23	

n: *the number of the samples*

At the end of the trials on the effect of self-pollination on seed production, no fruit formation was observed in the plant.It was calculated that *C. tomentosa* was distributed in an area of 28.70 km^2 and that the average number of individuals and the number of potential individuals in 5 populations were 16.56 ± 6.41 and 4,753,713 respectively. On the other hand, it was determined that populations with the largest size and number of individuals were in Akçakonak-Priene and National Park, South of Doğanbey respectively (Table 7).

Table 7. Size and number of individuals of *C. tomentosa* populations in different of populations

Name of the population	The number of sample areas	The average number of individuals	Size of the population per km²	The number of potential individuals
Meryem Ana	7	16,71±6,57	3,99	667,397
National Park- South of Doğanbey	17	18±7,39	6,00	1081,440
National Park- North of Doğanbey	6	13,71±6,57	1,14	155,608
Çamlık - Ortaklar	7	12,85±5,63	3,08	396,936
Akçakonak -Priene	13	19,57±6,85	14,49	2,833,736
Total	**50**	**16,56±6,41**	**28,70**	**4,753,713**

4. Conclusions and discussion

Our floral morphology observations revealed that the pollen presentation occurred during the opening of the buds, that the full pollen presentation occurred in fully opened flowers, that the stigma lobes enlarged after the pollen presentation and that the stigma lobes were closed before the pollen presentation. On the other hand, our morphological measurements demonstrated that the length of the styles which was 16.52 mm during the pollen loading phase increased by 8.26 mm and reached 24.78 mm during the pollen presentation phase (Table 2). It was reported that *Campanula* flowers were protoandric, that pollen grains were loaded before the enlargement of the stigma lobes, and that although not always, pollen grains were dispersed during the budding phase or opening of the buds [19]. Researchers have reported that pollen grains are rapidly released when anthers open during the budding stage of *C. bononiensis* flowers and that all the pollen grains released are presented on the styles when the corolla is opened. They have also reported that the pollen presentation in *C. bononiensis* flowers is extended by about 2 days per flower due to the secondary pollen presentation. On the other hand, it was reported that in protandric species, the prolongation of the male phase was considered as an advantage based on phenology, and the ovules maturated rapidly during the flowering period [16].

In our studies on *C. tomentosa*, it was determined that the stigmas of flowers were not functional during the pollen loading phase, and that completely curled stigmas were functional only during the pollen presentation phase. However, the percentage of viability of the pollen grains belonging to the flowers decreased in this phase. These results were supported by pollen and stigma viability tests (Table 4), which is undoubtedly a mechanism that prevents self-pollination and encourages external pollination. While fruit formation does not occur in *C. tomentosa* buds closed during the budding phase, the presence of proterandry suggests that pollination is totally dependent on a pollinator. It has been reported that members of the genus *Xylocopa* display polylectic behaviors and that they prefer large, fancy flowers with a lot of pollen and nectar [17]. Our observations also demonstrated that *X. valga* (Gerstacker, 1872) was one of the major pollinators of *C. tomentosa*, but did not visit other *Campanula* taxa with much smaller flowers probably due to their body size. The duration of *X. valga'*s visit (Gerstacker, 1872) (17.5 ± 4.68 sec) is shorter than that of *E. setulellus* (Strand, 1909) (Figure 2). However, according to the floral morphology data of *C. tomentosa*, the average diameter of the corolla tube is 34 mm and the diameter of the stigma in the middle of this tube is 5.6 mm, and the body size of the *X. valga* (15x30 mm) is greater, which clearly indicates that their contact with the stigma is essential at every time they visit flowers (Table 1; Figure4). In addition, the fact that the body structure of *X. valga* is more hairy than that of *E. setulellus* (Strand, 1909) (according to comparison of museum samples) also leads to a more effective pollination of the plant.

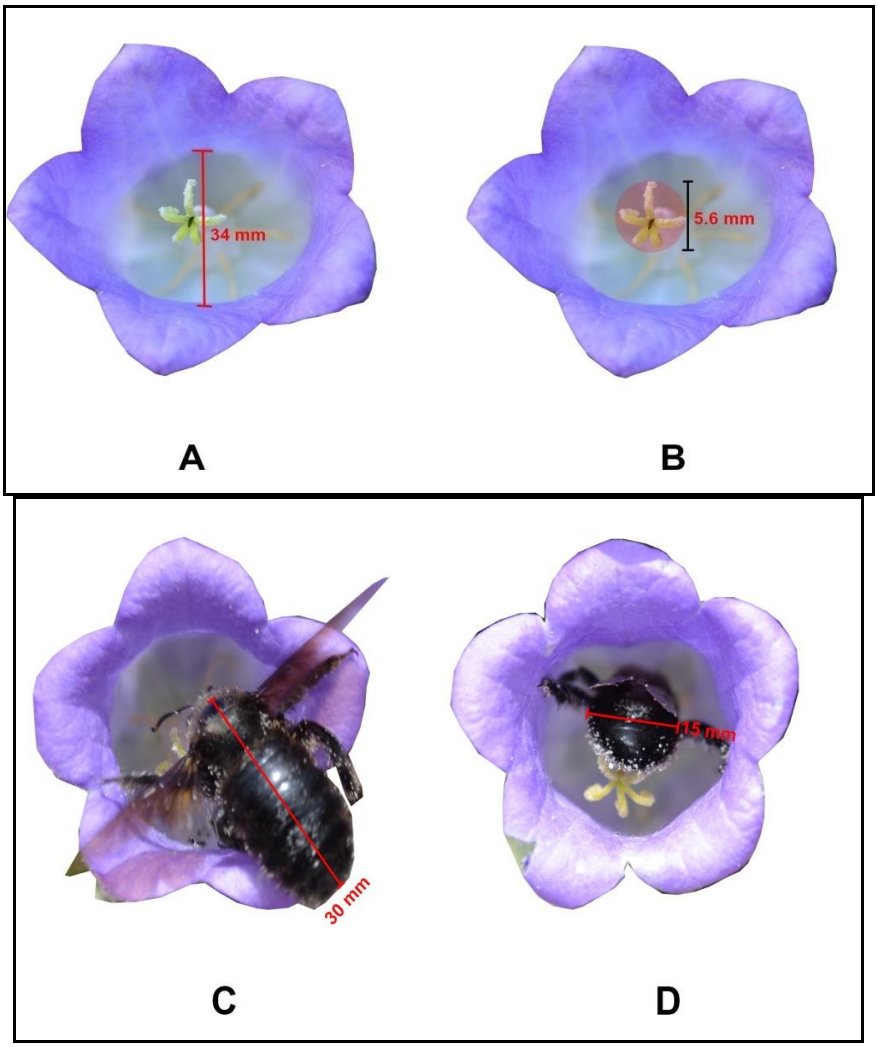

Figure 4. Morphological values in *C. tomentosa* and *X. valga*;
A: Corolla diameter; **B:** Stigma diameter; **C** and **D:** *X. valga*'s body size values.

As reported by researchers, when the main pollinator (Bombus spp.) of *Campanula punctata* enters its corolla to collect nectar, there will be an interaction between the dorsal surface of its chest and the style provided that there is a good match between the pollinator and flower [18], and size matching between the style and pollinator is expected to greatly affect the efficiency of pollination since the style of *C. punctata* has both male and female functions. In the same study, it was also reported that the duration of the visit by *Bombus* would significantly affected the accumulation of pollen on the stigma. The same researchers also reported that during their flower visits, the Bombus bees change their position around the corolla to insert their galea, which is a relatively solid organ, into the nectar spaces. It has been reported that the prolongation of this type of hunting behavior will contribute to the increase of pollen accumulation on the stigma. However, it was reported that although members of Megalchilide were locally important pollinators of *Campanula*, Halicutidae and Bombus bees were much more effective [19]. The same researchers also determined the mating and sleeping patterns of diptera species and of ants in the flowers of this species. *Campanula* flowers have a unique secondary pollen presentation. In this system; introrse anthers which dehisce inward before anthesis disperse pollen grains on the pollen-gathering feathers of the style [20]. At the beginning of the anthesis, all the pollen grains stick on the style, and the stigma lobes are yet neither opened nor functional. Another study reported that, flower visitors are oriented toward the stigma from the base of the style because the pollen tufts lean towards the style at this stage. Within a few days, as the stigma lobes are opened, the stigma becomes functional and accepts pollen grains. In the species we worked on, it was found that the hair on the style leaned towards the stigma from the base of the style [21]. When the average sugar concentration and sucrose measurements from the flowers of *C. tomentosa* and the changes in temperature-humidity and visit numbers during the day are examined, (Figure3), although the nectar concentration increased in the early evening hours, the number of visits to *X. valga* gradually decreased due to the increase in temperature (decrease in the humidity) (Figure3). Bees are ectothermic like other insect taxa and must protect their body temperature while flying. The thermal properties of their surroundings are important in their pollination activities. The high surface-to-volume ratio of small bees ensures rapid absorption of heat at high ambient temperatures and rapid cooling at low ambient temperatures. All the bees having a body mass greater than 35-50 mg

can produce endothermic heating, in other words internal heat production [22]. Examples of bee pollinators having a body weight of greater than 35 mg are Apis, Bombus, Xylocopa and Megachile. Examples of small bee pollinators are found in the Halicidae family, including the genus Lasioglossum. Many bees, in addition to endothermy, can control their temperatures before, during and after flying via physiological and behavioral ways. Endothermic abilities and thermal requirements vary between different groups of bees [23]. While *Xylocopa pubescens* generally cannot display nutritional activity at temperatures lower than 18°C, more thermophilic *X. sulcatipes* and *X. capitata* show nutritional activity when the ambient temperature reaches 22-32°C [24]. Nectar sugar concentration is mainly associated with the solar irradiance and temperature and is less associated with the bee activity. It was observed that *C. tomentosa* nutrition activity started at temperatures above 19°C, but that flower visits decreased as the temperature increased and that there were no visits after the temperatures reached 27°C (Figure3, 4). Given the reproductive parameters in the present study, it was determined that 93.42% of *C. tomentosa* flowers turned into fruit and that the Seed Setting Rate (SSR) in these fruits was 73.12%. The possible cause of this decline in seed setting rates can be said to be associated with the pollinators' behaviors. These results are considered to be an important parameter of the limited distribution.

Researchers report that *C. tomentosa* is distributed at rock formations at elevations of 100-130 meters located across from the cement plant in Söke, a district of Aydın, Türkiye [25]. However, in our field studies, *C. tomentosa* was not distributed anywhere in Söke, except for. Akçakonak-Priene, which suggests that there was a decrease in the number of distribution localities of *C. tomentosa*. In our field studies, compared to the previous year, a decrease was observed in the number of mature individuals of *C. tomentosa* in the north of the National Park, especially in the rocky areas near the entrance of the canyon. These significant habitat losses in *C. tomentosa* population is thought to be caused by the activities of Akçakonak stone crushing plant located in Söke-Akçakonak. In another study, *C. tomentosa* was classified as VU according to the IUCN Red List Categories (2001) [13]. However, when all populations of the plant were considered in the present study, approximately 4,753,713 potential individuals were identified in an area of 28.7 km². According to this, it was observed that the area occupied by all populations was less than 500 km^2 (B2). It was also determined that the habitat quality (b, iii), the number of distribution localities (b, iv) and the number of mature individuals (b, v) decreased. *C. tomentosa* was classified as EN B2b (iii, iv, v) (Endangered) according to IUCN 2001v3.1 (the Red List Categories and Criteria version 3.1, 2001).

Acknowledgements

The present study was supported by Ege University Rector Scientific Research Project Office (Project No: 2012- FEN-019), the Scientific and Technological Research Council of Türkiye (TUBITAK Project No: 112T598) and Science, Technology Application and Research Center of Ege University (EBILTEM Project No: 2013 / BİL / 017).

We would like to extend our sincere thanks to Dr. Fatih DİKMEN, Hacettepe University Science Faculty Biology Department Zoology Department for his valuable contributions to the identification of pollinators.

References

[1] Nabhan, G. P. & Buchmann, S. L. (1997). Services provided by pollinators. In: Daily GC (Eds.), Nature's Services: Societal Dependence on Natural Ecosystems (pp.133-150). Washington, D.C.: Island Press.

[2] Kelber, A., Warrant, E. J. & Pfaff, M. (2006). Light intensity limits foraging activity in nocturnal and crepuscular bees. *Behavioral Ecology*, 17, 63-72. *https://doi.org/10.1093/beheco/arj001*

[3] Cronquist, A. (1988). The evolution and classification of flowering plants (2nd ed.). Bronx, NY: The New York Botanical Garden Press.

[4] Caser, M., Scariot V. & Arens, P. (2010). Consequences of Geographical Habitats on Population Structure and Genetic Diversity in *Campanula* spp.. *International Journal of Plant Biology*, *https://doi:10.4081/pb.2010.e5*.

[5] Alsos, I. G., Engelskjøn, T. & Brochmann C. (2002). Conservation genetics and population history of *Betula nana*, *Vaccinium uliginosum* and *Campanula rotundifolia* in the arctic Archipelago of Svalbard. *Arctic, Antarctic, and Alpine Research*, 34, 408-418. *https://doi: 10.1080/15230430.2002.12003511*

[6] Denisow, B., Wrzesień, M., Bożek, M., Jeżak, A. & Strzałkowska-Abramek, M. (2014). Flowering, pollen characteristics and insect foraging on *Campanula bononiensis* (Campanulaceae), a protected species in Poland. *Acta Agrobotanica*, 67(2): 13-22. *https://doi: 10.5586/aa.2014.021*

[7] Damboldt, J. (1976). Materials for a flora of Türkiye 32: Campanulaceae. Notes from the Royal Botanic Garden, Edinburgh 35: 39-52.

[8] Yıldırım, H. & Şenol, S. G. (2014). *Campanula alisan-kilincii* (Campanulaceae), a new species from eastern Anatolia, Türkiye, *Turkish Journal of Botany*, 38, 22-30. *https://doi: 10.3906/bot-1302-17*

[9] Alçıtepe, E. (2010). Studies on seed morphology of *Campanula* L. Section Quinqueloculares (Boiss.) Phitos (Campanulaceae) in Türkiye, *Pakistan Journal of Botany*, 42(2), 1075-1082.

[10] Subaşı, Ü. & Güvensen, A. (2014). Seed germination studies on chasmophyte endemic *Campanula tomentosa* and *Campanula vardariana*. *Biological Diversity and Conservation*, 7(2), 129-135.

[11] Subaşı, Ü., Şenol, S.G., Eroğlu, V., Güvensen, A. & Seçmen, Ö. (2012). Seed germination and conservation of rare endemic *Campanula teucrioides* Boiss. (Campanulaceae). "The Second International Symposium on the Biology of Rare and Endemic Plant Species (Biorare-2012)", April 24-27, Fethiye, Muğla, Türkiye.

[12] Gücel, S. & Seçmen, Ö. (2008). Reproductive biology of subalpin endemic *Minuartia nifensis* Mc Neill (Caryophyllaceae) from West Anatolia, Türkiye. *Biological Diversity and Conservation*, 1(1), 66-74.

[13] IUCN (2001). Species survival commission, IUCNRed List Categories and Criteria: Version 3.1., Gland, Switzerlandand Cambridge, UK, 30p.

[14] Galetto, L. & Bernardello, G. (2005). Rewards in flowers: nectar. In: Dafni A., Kevan P.G., Husband B.C. (Eds), Practical pollination biology (pp. 261-313). Ontario, Canada.-Enviroquest.

[15] Dafni, A. (1992). Pollination Ecology: A practical approach. Oxford University Press, Oxford, 250p.

[16] Shetler, S. G. (1979). Pollen-collecting hairs of *Campanula* (Campanulaceae). *I. Historical review*, *Taxon*, 28, 1-3), 205-215.

[17] Obeso, J. R. (2002). The costs of reproduction in plants. *New Phytologist*, 155, 321–348. *https://doi: org/10.1046/j.1469-8137.2002.00477.x*

[18] Hurd, P. D. (1955). The carpenter bees of California (Hymenoptera: Apoidea). Bulletin of the California insect survey. University of California Press.

[19] Inoue, K. & Amano, M. (1986). Evolution of *Campanula punctata* Lam. in the Izu Islands: Changes of pollinators and evolution of breeding systems. *Plant Species Biology*, 1(1), 89-97.

[20] Inoue, K. (1990). Dichogamy, sex allocation, and mating system of *Campanula microdonta* and *C. punctata*. *Plant Species Biology*, 5, 197–203.

[21] Yeo, P. F. (1993). Secondary pollen presentation. Form, function and evolution. *Plant Systematic and Evolution*, Wien New York, Springer.

[22] Leins, P. (2000). Blüte und frucht. morphologie, entwicklungsgeschichte, phylogenie, funktion, Okologie. E. Schweizerbart' sche Verlagsbuchhandlung, Stuttgart.

[23] Bishop, J. A. & Armbruster, W. S. (1999). Thermoregulatory abilities of Alaskan bees: Effects of size, phylogeny and ecology. *Functional Ecology*, 13, 711-724

[24] Gerling, D., Velthuis, H. H. W. & Hefetz, A. (1989). Bionomics of the large carpenter bees of the genus Xylocopa. *Annual Review of Entomology*, 34, 163–190.

[25] Alçıtepe E. & Yıldız K. (2010). Taxonomy of *Campanula tomentosa* Lam. and *C. vardariana* Bocquet from Türkiye. *Turkish Journal of Botany*, 34, 191–200.