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ORIGINAL PAPER

Flower structure and floral reward in *Scopolia carniolica* (Solanaceae) – is it a plant that can support the bumblebee food base in early spring?

Karolina Tymoszuk[1] · **Marta Dmitruk**[2] · **Jacek Jachuła**[1] · **Bożena Denisow**[2]

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Abstract

Scopolia carniolica Jacq. is a perennial plant from the family Solanaceae. The study objective was to examine floral traits that may be important for interactions with floral visitors (i.e., blooming phenology, floral micromorphology, nectary characteristics, nectar quantity, nectar sugar composition, and pollen production). In *S. carniolica*, papillae, numerous non-glandular trichomes, and a few glandular trichomes were present on the inner corolla epidermal surface. Lipids, acidic lipids, tannins, and alkaloids were present in the non-glandular trichomes and in the corolla cells. The discoid-type nectary was located at the base of the ovary. Floral nectar was released through nectarostomata. The process of nectar release started in the bud stage (ca. 5–8 h before corolla opening) and continued to the 4th day of anthesis. The amount of secreted nectar peaked in 2-day-old flowers. The amount of produced nectar, nectar sugar concentration, and sugar mass varied significantly across years. On average, the total mass of sugars in the nectar was 0.54 mg/flower. *S. carniolica* produced sucrose-dominant nectar with no glucose. The sugar proportions did not differ during the flowering season. On average, 1.95 mg of pollen per flower was produced. Among floral visitors, bumblebees were most frequently noted, accounting for 79.7% of the total number of floral visitors to *S. carniolica* flowers. The species can be used in early spring ornamental arrangements to support the food supply for insects, mainly bumblebees.

Keywords Floral trichomes · Nectary · Floral nectar · Nectar carbohydrates · Pollen · *Bombus* spp

Introduction

Scopolia carniolica Jacq. is a perennial plant from the family Solanaceae (APG system, Ravel system). The species is distributed throughout south-eastern Europe, and its range extends to the Caucasian region (Lonati and Siniscalco 2009). *S. carniolica* has also been naturalized in other countries (e.g., Germany, Denmark) and is cultivated due to its medicinal properties (Grynkiewicz and Gadzikowska 2008). In Eastern Europe, populations of *S. carniolica* are associated with deciduous forests of the Querco-Fagetea class (Lonati and Siniscalco 2009). In Poland, the occurrence of *S. carniolica* is limited to the eastern part of the Carpathian Mountains, where the species is present in a small number of sites (Delimat and Walusiak 2009; Winnicki and Zemanek 2014). The species is now under partial protection in Poland. In the IUCN Red List of Threatened Species, *S. carniolica* was listed as Least Concern species (Khela 2013). *S. carniolica* plants have a height of approx. 40–60 cm and produce single bell-shaped flowers

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✉ Marta Dmitruk
marta.dmitruk@up.lublin.pl

✉ Bożena Denisow
bozena.denisow@up.lublin.pl

Karolina Tymoszuk
karolina.tymoszuk@mail.umcs.pl

Jacek Jachuła
jacek.jachula@mail.umcs.pl

[1] Present address: Department of Botany, Mycology and Ecology, Institute of Biological Sciences, Maria Curie-Sklodowska University, 19 Akademicka St, Lublin 20-033, Poland

[2] Department of Botany and Plant Physiology, University of Life Sciences in Lublin, 15 Akademicka St, Lublin 20-950, Poland

(15–25 mm) with a dark reddish-brown corolla (Delimat and Walusiak 2009).

Angiosperms use various attractants to lure pollinators (Wester and Lanau 2017). The flower size, shape, color, and odor are of great importance, as they can signal the absence or presence of floral reward (Antoń et al. 2012; Jachuła et al. 2018). However, a fundamental role in mediating plant-pollinator interactions is assigned to floral rewards, mainly nectar and pollen (Nicholls and Hempel de Ibarra 2017; Nepi et al. 2018). It is widely accepted that variation in the amount and rate of nectar production can affect the frequency of insect pollinators, duration of their visit to flowers, and consequently the pollination success and fitness of the species (e.g., Manetas and Petropoulou 2000; Bruinsma et al. 2014). In addition to nectar quantity, nectar traits (nectar viscosity and/or chemistry, e.g., sugar composition) have been reported to correlate with pollinator groups (e.g., Baker and Baker 1975; Martínez del Rio et al. 1992; Antoń and Denisow 2014; Roguz and Zych 2016).

Floral nectar is an aqueous solution of sugars (mainly disaccharides - sucrose, and hexoses - glucose and fructose) that provides energetic reward (e.g., Heinrich 1975; Dmitruk et al. 2022). Nectar may also contain low concentrations of protein enzymes, amino acids, minerals, and secondary metabolites (Nepi et al. 2012; Afik et al. 2014; Roy et al. 2017). The nectar sugar composition is species-specific and can be categorized based on the sucrose: hexose ratio (Baker and Baker 1983). Nectar characteristics (i.e., sugar concentration, sugar proportions) may vary among individuals (Canto et al. 2011; Antoń et al. 2017), flowers (Nicolson et al. 2007), or even floral phases (Cruden et al. 1983; Langenberger and Davis 2002; Carlson 2007). Moreover, nectar traits (in particular sugar concentration and nectar volume) are very sensitive to environmental variations across the floral life-span (e.g., Petanidou and Smets 1996).

Pollen is a basic protein food for insect pollinators (Leonhardt and Blüthgen 2012; Ruedenauer et al. 2016). Additionally, it contains lipids, sterols, vitamins, and hormones (Roulston et al. 2000; Filipiak et al. 2017; Dmitruk et al. 2023). Pollen is crucial in the pollinator diet, and its diversity is essential to ensure nutrients required for their development and proper behavior (Filipiak et al. 2017). Pollen diet also affects pollinator resistance to diseases (Palmer-Young et al. 2017; Parreño et al. 2021).

In the temperate zone of the Northern Hemisphere, early spring (March-April) is a period when insect pollinators may experience food scarcities (Ogilvie and Forrest 2017; Jachuła et al. 2021). Bumblebee queens create new colonies each year and the availability of nectar and pollen at the beginning of the bumblebee activity season has a strong effect on the number of established nests and colony performance (Timberlake et al. 2019; Requier et al. 2020). The ground layer of woodlots, forests and urban parks offers important floral resources for pollinating insects in early spring and can be supplemented with forage species to support particular groups of pollinating insects (Dylewski et al. 2020). However, to choose the best plant species to help pollinators, the quantity and quality of nectar and pollen produced should be recognized (Jachuła et al. 2019; Filipiak et al. 2022).

This research aimed to examine the features of *S. carniolica* flowers that may be important for floral visitors. In particular, the study was focused on (i) blooming biology (phenology, anthesis length), (ii) features of floral micromorphology (e.g., the distribution of trichomes on the corolla), (iii) nectary location and structure, (iv) quantity and nectar sugar composition, (v) amount of produced pollen and protein content, and (vi) insect visitor composition.

Materials and methods

Study site and study population

The study was carried out in 2017–2019 in the Botanical Garden of Maria Curie-Skłodowska University (51° 16' N, 22° 30' E, 200 m a.s.l.) in Lublin, Poland. The population of *S. carniolica* was imported from the Bieszczady Mountains (SE Poland) and established in the Garden in the 1970s. According to the inventory book of the Botanical Garden, it originated from two populations. The first is under inventory number 877, dated 1973, from Zasan, Bieszczady (49°23'N 22°25'E) and the second is under number 2166, dated 1976, from Bukowe Berdo, Bieszczady (49°07'N 22°41'E). Currently, the population covers an area of approx. 20 m^2.

The study site is composed mainly of species associated with the communities of the *Fagetalia sylvaticeae* order (e.g., *Fagus sylvatica* L., *Ranunculus cassubicus* L., *Symphytum cordatum* L., *Allium ursinum* L., *Galium odoratum* (L.) Scop., *Lilium martagon* L. and *Lunaria rediviva* L.).

Flowering and flower development

The onset and duration of the successive stages of flowering were recorded according to the protocol described in detail by Denisow (2009). The phenological phases were defined as follows: the initiation of blooming = when approx. 10% of flowers started to bloom, the full blooming phase = when > 50% of flowers were in bloom, and the end of blooming = when > 70% of flowers had wilted corollas. To assess the flower life-span (time between flower opening and corolla wilting), 15 flowers were marked at the bud stage each year and observed every two hours from 8.00 to 18.00 (GMT + 2 h).

Flower morphological traits (corolla length and diameter) were measured with a digital caliper to the nearest 0.01 mm, at randomly selected flowers ($n = 30$).

Microscopic observations

In 2019, the floral microstructure was observed in flowers that were 1–4 days old using bright-field (BFM)- fluorescence (FLM), and scanning electron microscopy (SEM). The flowers for microscopic observations were randomly collected from different plants ($n = 10$). The structure of floral nectaries was studied in nectar-bearing flowers on the 2nd day of anthesis.

Bright-field microscopy (BFM)

First, the position of the nectary and the trichomes in fresh flowers was determined using an Olympus SZX12 (Tokyo, Japan) stereomicroscope. The distribution of nectaries and trichomes in fresh flowers ($n = 10$) was investigated. The height of epidermis and parenchyma cells of nectaries ($n = 10$) and the length of trichomes ($n = 30$; from the base of the trichome to its apex) were measured.

Floral nectaries (both in the bud stage and in open flowers) were fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.4; 0.1 M) for 12 h at 4 °C and washed three times in phosphate buffer. They were then post-fixed in a 1% osmium tetroxide solution for 1.5 h at 0 °C and washed three times in distilled water. Subsequently, the fixed material was dehydrated in a graded ethanol series and infiltrated with LR White Resin (LR White acrylic resin, medium grade, Sigma-Aldrich). Following polymerization at 60 °C, semi-thin sections were cut at 0.6–0.9 µm with a glass knife for light microscopy using a Reichert Ultracut-Ultramicrotome (Leica, Vienna, Austria). For general histology, the semi-thin sections were stained with 1% (w/v) 1:1 aqueous methylene blue: azure II (Gahan 1984). The presence of insoluble polysaccharides was tested with the Periodic acid-Schiff's (PAS) reagent after blocking free aldehyde groups. The sections were examined by means of a Nikon Eclipse E400 (Tokyo, Japan) light microscope.

Fresh hand-made sections of corollas with trichomes were tested using the following histochemical tests: Nile Blue for neutral and acidic lipids (Jensen 1962), Sudan Red for total lipids (Brundrett et al. 1991), potassium dichromate for tannins (Gabe 1968) and Wagner's reagent for alkaloids (Adler 2000). After every reaction, the plant material was washed in distilled water and mounted in a 50% aqueous solution of glycerol (Łotocka 2023). The stained sections were observed and photographed with a Nikon Eclipse 400 (Tokyo, Japan) light microscope.

Fluorescence microscopy (FM)

For the fluorescence microscopy investigations, fresh material was treated with 10% aluminum trichloride for flavonoids (Charrière-Ladreix 1976). Autofluorescence was tested in fresh hand-cut sections of the corolla with trichomes, and the nectary illuminated with UV light. The observations were conducted using a Nikon 90i fluorescence microscope with a digital camera (Nikon Fi1) and NIS-Elements Br 2 software.

Scanning electron microscopy (SEM)

For observations in SEM, flower fragments were fixed in 4% glutaraldehyde in phosphate buffer (pH 7.4; 0.1 M) at 4 °C. Then, the material was washed in phosphate buffer, dehydrated in a graded acetone series, and subjected to critical-point drying using liquid CO_2. Next, the material was sputter-coated with gold using an EMITECH K550x sputter coater. Nectaries and other flower microstructures were observed and documented in a TESCAN VEGA II LMU SEM microscope (Brno, Czech Republic) at an accelerating voltage of 30 kV.

Floral reward

Nectar was collected using micropipettes. Prior to sample collection, the flowers were covered with tulle isolators to prevent access by insects. To assess the dynamics of nectar sugar accumulation, the nectar was collected at five time points, i.e., (i) in the bud stage (1–2 h before flower opening) and in 1-day, 2-day, 3-day, and 4-day old flowers. At each time point, nectar was collected from 10 randomly chosen flowers selected from different individuals. For each year, 3 replicates were done. In total, the nectar from 450 flowers was analyzed. The sugar concentration in the nectar was measured with an automatic refractometer (Rudolph Research Analytical, model J-357). The mass of produced sugars was calculated per flower.

To determine the mass of produced pollen, the ether-ethanol method described in detail by Denisow (2011) was used. Each year, unopened mature anthers ($n = 20$) collected from randomly selected flowers were transferred into glass containers ($n = 5$) with known masses and dried in an ELCON CL 65 dryer (35 °C, 7 days). Ether (2 ml) was poured on the anthers and left to evaporate. Then, pollen was rinsed from the anthers using 2–5 ml of 70% ethanol 3–5 times and dried and the containers were reweighed. Pollen production was calculated per flower.

To determine the composition of nectar carbohydrates, high-performance liquid chromatography (Shimadzu HPLC system) with a refractive index detector was engaged. The

system included an LC-10ADVP pump, CTO-10ASVP column oven, SIL-10ADVP auto-injector, PhenoSphere LC column, NH2 80 Å, 250 × 4.6 mm (Phenomenex Inc., Torrance, CA, USA) and RID-10 A detector. Elution of the carbohydrates was carried out using a mobile phase 80:20 v/v acetonitrile: water at flow rate 1.5 mL/min, column and detector temperature = 30 °C, injection volume = 20 µL. The mean amount of the carbohydrates was calculated from the samples and expressed as a percentage of total sugars. Then, the sugar ratio (r) was determined (r = sucrose/(glucose + fructose): S/(G + F) (Baker and Baker 1983; Nicolson and Thornburg 2007). According to the detailed observations on flower development, two sexual phases can be distinguished: in the first day of flower life-span the anthers are closed (= female phase), and from the second day of flower life-span the anthers start to dehisce (= male phase). Therefore, the HPLC analyses were made separately for female phase (1-day old flowers) and male phase (2–3 day old flowers). For each phase, a sample of nectar pooled from 12 to 15 flowers (each flower from different shoot) was analysed in triplicate (3 biological × 3 technical replicates).

The protein content in the *Scopolia carniolica* pollen was recorded with the protein Bradford method (Bradford 1976). The measurements were made using NanoPhotometer® N60 (Kyoto, Japan) with a 1.0 nm scan pitch and a 190–850 nm scan range in 60s. The pollen samples were first stirred, and the assay was performed at room temperature. The zero standard was prepared with Bradford reagent and ddH2O.

Insect visitors

The spectrum and abundance of insect visitors were recorded during full bloom each year. This part of the study was carried out in 5 randomly selected plots of 1 m^2. The observations were made for 10 min at one-hour intervals from 8:00 a.m. to 6:00 p.m. (GMT + 2 h) for 5 consecutive days. The insects were identified to the lowest possible taxa. Due to difficulties in distinguishing *Bombus terrestris* from *B. lucorum*, *B. mangnus* and *B. cryptarum* in field conditions, the species are described in this paper as '*Bombus terrestris* group'.

Table 1 Blooming phenology and flower life-span in *S. carniolica* in 2017–2019 in Lublin, SE Poland

Year	Blooming period (days)	Full bloom period (days)	Flower life-span (days)
			mean ± SD
2017	28 March – 20 April (24)	12.0	$3.7 \pm 0.2_b$ ($n = 15$)
2018	07 April – 25 April (19)	12.0	$3.1 \pm 0.5_a$ ($n = 15$)
2019	16 April – 07 May (22)	11.0	$3.0 \pm 0.3_a$ ($n = 15$)
Mean	21.7 ± 2.1	11.7 ± 0.5	3.3 ± 0.3

Means with the same letter do not differ significantly between study seasons according to Tukey HSD test at α = 0.05; SD– standard deviation

Statistical analyses

Statistica 13.3 was applied for all calculations. Prior to any test, the data were checked for normality. Since the assumptions of normal distribution were not met, the data on the mass of sugars/flower were subjected to square root transformation. One-way analysis of variance (ANOVA) was used to test the significance of differences between the mean values of the analyzed features. When applicable, Tukey's HSD test was used for post hoc comparison of means at α = 0.05.

Results

Flowering

The flowering period of *Scopolia carniloca* differed between the years of the study (Table 1). The start of flowering was recorded in March/April, whereas the end was noted in April/May. The population flowered for 19–24 days, i.e., on average 21.7 days. The single flowers grew in the base of leaves on long drooping peduncles. The number of flowers ranged from 3 to 11 per shoot (mean = 6.4). The flower life-span ranged from 2.5 to 4.5 days (mean = 3.3 days) and was significantly different among years ($F2,42 = 24.732$, $p < 0.001$).

Flower micromorphology and nectary

S. carniolica (Fig. 1.A) produced flowers with fused petals forming a campanulate corolla tube (mean length 21.5 ± 1.3 mm and mean diameter 9.1 ± 1.4 mm; $n = 30$; Fig. 1. B-C). Actinomorphic flowers of *S. carniolica* were perfect and contained five anthers and one five-carpellate pistil. In the bud stage, the anthers remained closed (Fig. 1.D). During flower maturation the filaments elongated and the anthers gradually dehisced (Fig. 1.E-F). The epidermal surface of the flower corolla was covered by papillae (mean length 14.49 ± 2.84 µm; ranged 10.43 –19.13 µm; $n = 30$ and mean diameter 27.25 ± 2.72 µm; ranged 24.35 µm 31.30 µm; $n = 30$) (Fig. 2.A).

The inner surface of the corolla was covered with a few glandular (Fig. 2. B-D) and numerous non-glandular trichomes (Fig. 2.E-G). Two types of non-glandular trichomes were found: (i) short 1- or 2-celled and (ii) long 2- or 5-celled (Fig. 2. E). The length of non-glandular trichomes ranged from 40.92 µm to 664.95 µm (mean = 261.75 µm, ±SD 179.63; $n = 30$). The presence of lipids, acidic lipids,

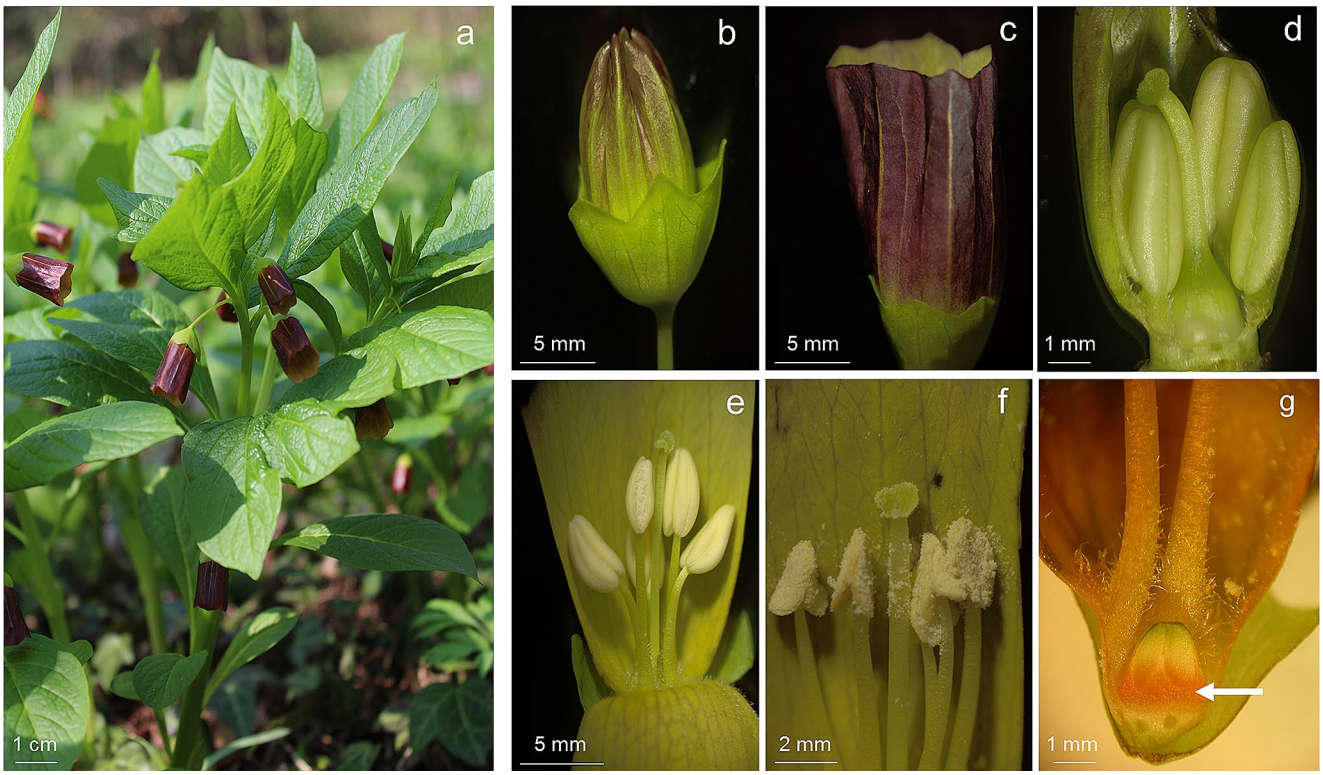

Fig. 1 Macrophotographs of *Scopolia carniolica*: (**a**) plants in the experimental population, (**b**) flower bud, (**c**) mature flower, (**d**) longitudinal section through flower bud, anther not dehisced, (**e**) longitudinal section through 2-day flower, beginning of pollen release, (**f**) longitudinal section through 3-day flower, all anthers releasing pollen, (**g**) longitudinal section of the ovary with the nectary at the base (arrow)

tannins, and alkaloids was detected in the non-glandular trichomes and in the corolla epidermal cells (Fig. 2. H-L). Flavonoids were detected in the corolla cells, whereas the non-glandular trichomes contained essential oils.

Nectary structure and anatomy

The nectary in the *S. carniolica* flowers was identified as the discoid type and was located at the base of the ovary (Fig. 3.A, G). Initially, in the bud stage, the nectary was light yellow. In turn, the nectary was yellow-orange in the open flower on the following days of anthesis (Fig. 1.G).

The nectary consisted of a single-layered epidermis and 5–7 layers of subepidermal parenchyma cells (Fig. 3. B-C). On average, the epidermal cells were 19.15 µm in height ($n = 10$). The outer walls of the epidermal cells were relatively thick and were covered with striated cuticular ornamentation which stained intensely in the autofluorescence assay (Fig. 2.L, 3.D). The cells of the subepidermal parenchyma layer were small (mean = 15.66 µm in height; $n = 10$). However, the parenchyma cells in the deeper layers of the nectary were larger (mean = 27.30 µm in height; $n = 10$). The nectary was supplied by both xylem and phloem elements located at its base. Staining with PAS revealed the presence of numerous starch grains in the plastids of young secretory parenchyma cells. Starch was evenly distributed in parenchyma cells (Fig. 3.E). At the time of intense nectar release, the epidermal cells were vacuolated (Fig. 3.C).

Floral nectar was exuded from nectarostomata, which were unevenly distributed in the upper epidermis of the nectary. Before the process of nectar secretion began, the nectarostomata were located below the epidermal cells (Fig. 3.F). However, at the time of nectar release, the nectarostomata were raised slightly above the level of the epidermis layer (Fig. 3.G-H). The stomatal complex was composed of two pore-forming guard cells and 5–6 adjacent subsidiary cells (Fig. 3.F-H).

Floral nectar and pollen

The floral nectar was accumulated at the bottom of the corolla. The process of nectar release started in the bud stage (approx. 5–8 h before corolla opening) and continued through the flower life-span, i.e., to the 4th day of anthesis (Fig. 4.). The flower age had a significant effect on the mass of the nectar secreted per flower ($F_{4,40} = 102.953$; $p < 0.001$), the sugar concentration in nectar ($F_{4,40} = 71.858$; $p < 0.001$), and the mass of produced sugars per flower ($F_{4,40} = 169.141$; $p < 0.001$). The amount of secreted nectar and the concentration of nectar sugars increased during the

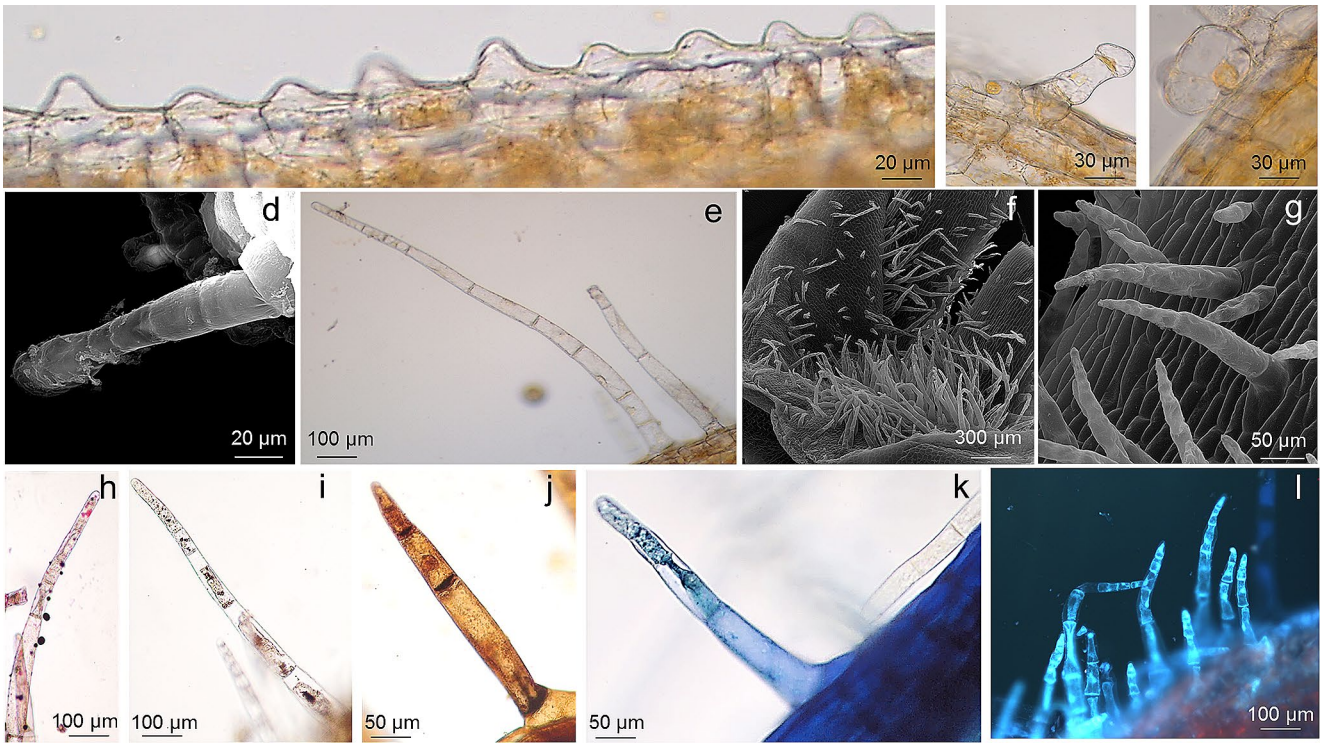

Fig. 2 Micromorphology of flower corolla: (**a-c**, **e**, **h**, **i-k**) BFM; (l) FM; (**d**, **f**, **g**) SEM; (**a**) papillae on the epidermal surface, (**b-d**) glandular trichomes (**e-g**) non-glandular trichomes (**h-k**) histochemistry of non-glandular trichomes: (**h**) lipids after Sudan Red staining (**i**) tannins after potassium dichromate staining (**j**) alkaloids after Wagner's staining (**k**) neutral and acidic lipids after Nile Blue staining, (**l**) fluorescence of flavonoids under UV light with aluminium trichloride

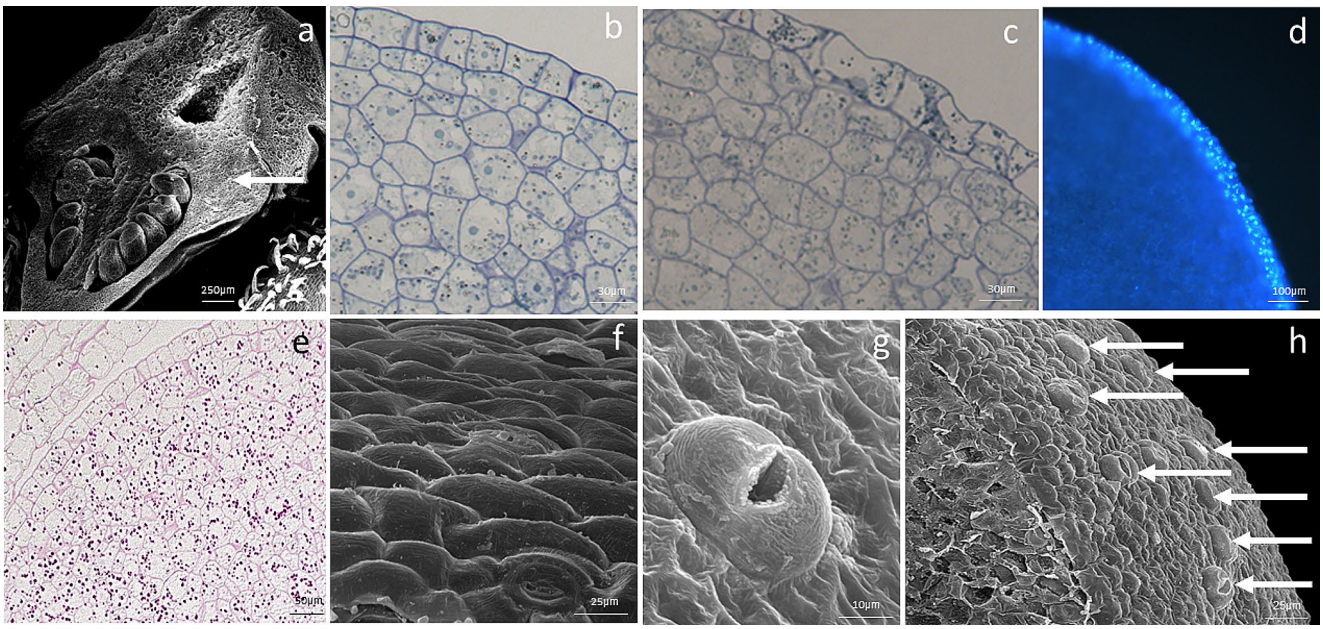

Fig. 3 Micromorphology of nectary: (**b**, **c**, **e**) BFM; (**d**) FM; (**a**, **f-h**) SEM; (**a**) location of nectary tissue (arrow) (**b**) nectary tissue in young flower (**c**) nectary tissue of mature flower (**d**) autofluorescence of epidermal nectary cells (**e**) starch grains in plastids of young secretory parenchyma cells after PAS staining, (**f**) nectarostomata before nectar secretion, located below the epidermal cells (**g**) nectarostomata secreting nectar, located above the epidermal cells (**h**) fragment of the nectary surface with nectarostomata (arrows)

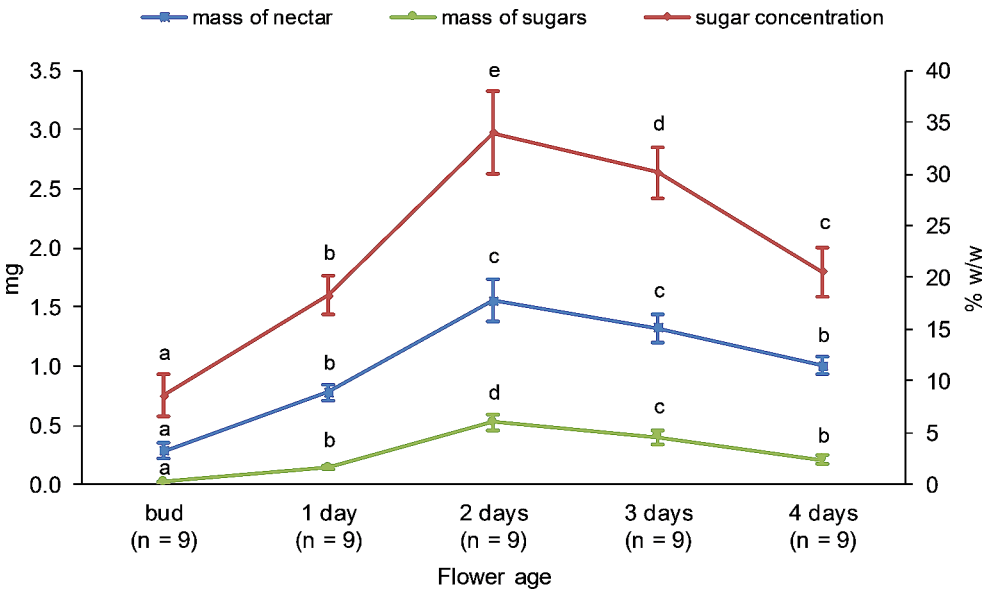

Fig. 4 Effect of flower age on the mass of nectar, nectar sugar concentration and the mass of sugars in *S. carniolica.* Values are means calculated across study seasons. Vertical bars show standard deviation. Means with the same letter do not differ significantly between flower age according to Tukey HSD test at α = 0.05

Table 2 Mass of nectar, concentration of sugars and mass of sugars per one flower in *S. carniolica* at the peak of nectar secretion (2-day flowers) in 2017–2019, Lublin, SE Poland

Year	Mass of nectar/1 flower (mg)	Nectar concentration (%w/w)	Mass of nectar sugars/1 flower (mg)
	mean ± SD	mean ± SD	mean ± SD
2017 (n = 3)	$2.09 \pm 0.27_b$	$28.6 \pm 1.6_a$	$0.60 \pm 0.17_b$
2018 (n = 3)	$1.88 \pm 0.10_b$	$32.8 \pm 3.6_{ab}$	$0.62 \pm 0.14_b$
2019 (n = 3)	$1.05 \pm 0.18_a$	$39.3 \pm 1.5_b$	$0.41 \pm 0.14_a$
Mean	1.67 ± 0.47	33.6 ± 4.2	0.54 ± 0.22

Means with the same letter do not differ significantly between study seasons according to Tukey HSD test at α=0.05; SD– standard deviation

first two days of flower anthesis. The values peaked in 2-day flowers. Next, the amount of secreted nectar and produced sugars decreased slightly towards the end of the flower lifespan (Fig. 4).

The nectar characteristics and their year-to-year variation tested on 2-day flowers are presented in Table 2. The nectar amount differed significantly among the years of the study ($F_{2,6} = 25.902$, $p = 0.001$). The amount of nectar produced per flower varied widely from 0.9 to 3.7 mg (on average 1.67 mg). Similarly, the year effect was found for the nectar concentration ($F_{2,6} = 11.679$, $p = 0.009$). The nectar concentration ranged from 26 to 42% (mean = 33.6%). Consequently, the total mass of sugar produced in the flowers differed significantly between the study seasons ($F_{2,6} = 5.874$, $p = 0.039$). On average, the total mass of sugars in the nectar was 0.54 mg/flower. The lowest total mass of sugars was detected in 2019; it was approx. 30% lower than in 2017 and 2018.

The nectar contained exclusively fructose (F) and sucrose (S). No other carbohydrates were identified in the nectar profile. A characteristic feature was the complete absence of glucose in the floral nectar of *S. carniolica*. On average, the nectar contained 66.67 ± 4.54% of sucrose and 33.33 ± 3.85% of fructose (Table 3). No significant differences were found for fructose and sucrose content between female and male phase.

The flowers of *S. carniolica* had five anthers with filaments adhering to the corolla. The anthers dehisced on the 2nd day of anthesis. No open anthers were observed in the bud stage or in 1-day old flowers. No significant year effect was found for the pollen production per flower ($F_{2,12} = 1.110$, $p = 0.361$). On average, 1.95 mg of pollen per flower was produced (Table 4).

The protein content in the pollen averaged 17.7 ± 3.0% (w/w).

Open flowers of *S. carniolica* were visited only by representatives of Hymenoptera. The insects foraged between 11.00 and 16.00; however, they were active mainly in mid-day hours from ca. 12.00 h to 14.00 h. Every year,

Table 3 Percentage (%) carbohydrate composition, sugar ratios (r), and hexose ratios (rh) in floral nectar from *S. carniolica*

Data represent mean values ± SD – standard deviation. Means with the same letter do not differ significantly between sexual phases according to Tukey HSD test at α=0.05

Sexual phase	Glucose (G)	Fructose (F)	Sucrose (S)	r = S/(G + F)	rh = G/F
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD
female (n = 3)	0.00 ± 0.00	$34.45 \pm 4.34_a$	$65.55 \pm 4.11_a$	$1.90 \pm 0.49_a$	0.00 ± 0.00
male (n = 3)	0.00 ± 0.00	$32.21 \pm 4.92_a$	$67.79 \pm 5.03_a$	$2.10 \pm 0.41_a$	0.00 ± 0.00
mean	0.00 ± 0.00	33.33 ± 3.85	66.67 ± 4.54	2.00 ± 0.36	0.00 ± 0.00

Table 4 Mass of pollen per 1 flower in *S. carniolica* in years 2017–2019

Year	Mass of pollen per 1 flower (mg)	
	min - max	mean ± SD
2017 ($n=5$)	1.66–2.73	$2.01_a \pm 0.68$
2018 ($n=5$)	1.37–2.26	$1.94_a \pm 0.62$
2019 ($n=5$)	1.37–2.57	$1.89_a \pm 0.37$
Mean		1.95 ± 0.56

Means with the same lowercase letter do not differ significantly between study seasons according to Tukey HSD test at α=0.05; SD– standard deviation

bumblebees were noted most frequently (Fig. 5). Their proportion ranged from 69 to 100% among the study seasons (mean = 79.7) Among bumblebees, *Bombus pascuorum* and representatives of *B. terrestris* group were observed to be most abundant in the flowers, accounting for 38.0% and 29.3% of all insect visitors, respectively. Individuals of *B. lapidarius* were noted as well (mean = 12.3%). The other insect visitors observed were *Andrena* species and other solitary bees.

Discussion

In the temperate zone of the Northern Hemisphere, *Scopolia carniolica* blooms in early spring, with the full bloom period in April. Although the amount of available nectar sugars is low, the species is a good pollen producer (discussed in detail below). Early spring food resources are critical for pollinators as in this period they build the nests (in particular bumblebee queens) and raise the next generations. During this period we identified few flowering species, e.g., tree and shrub species – *Salix, Acer, Prunus, Ribes* and herbaceous common weeds, including *Tussilago, Taraxacum. Lamium*. Even more nectar and pollen potential sources can be found in ornamental gardens e.g., *Crocus, Galanthus, Pulmonaria, Ficaria, Anemone.* Studies from diverse authors indicate that these resources are sufficient compared to requirements of pollinators (e.g., Timberlake et al. 2019; Jachuła et al. 2021). However, according to various recommendations, different nectar and pollen species should be cultivated to increase nutrient diversity necessary to support the development of healthy pollinators (Filipiak et al. 2017; McMinn-Sauder et al. 2022). In particular, the species with confirmed high availability of pollen should be selected to grant maximum advantage for pollinators in early spring (e.g., Denisow 2011; Filipiak et al. 2017; Jachuła et al. 2022; Dmitruk et al. 2023).

The campanulate corolla of *S. carniolica* is open to different insect visitors. However, the flowers were mainly visited by the species from the genus *Bombus*. The flowers (size = 20–25 mm in length; 7-10- mm in diameter) provide a landing platform for bumblebees. However, due to the corolla morphology, in particular long-tongue bumblebees can easily reach the nectar available inside at the base of ovary. The contrast between the interior (light yellow) and exterior (purple-brown) surface of the corolla may

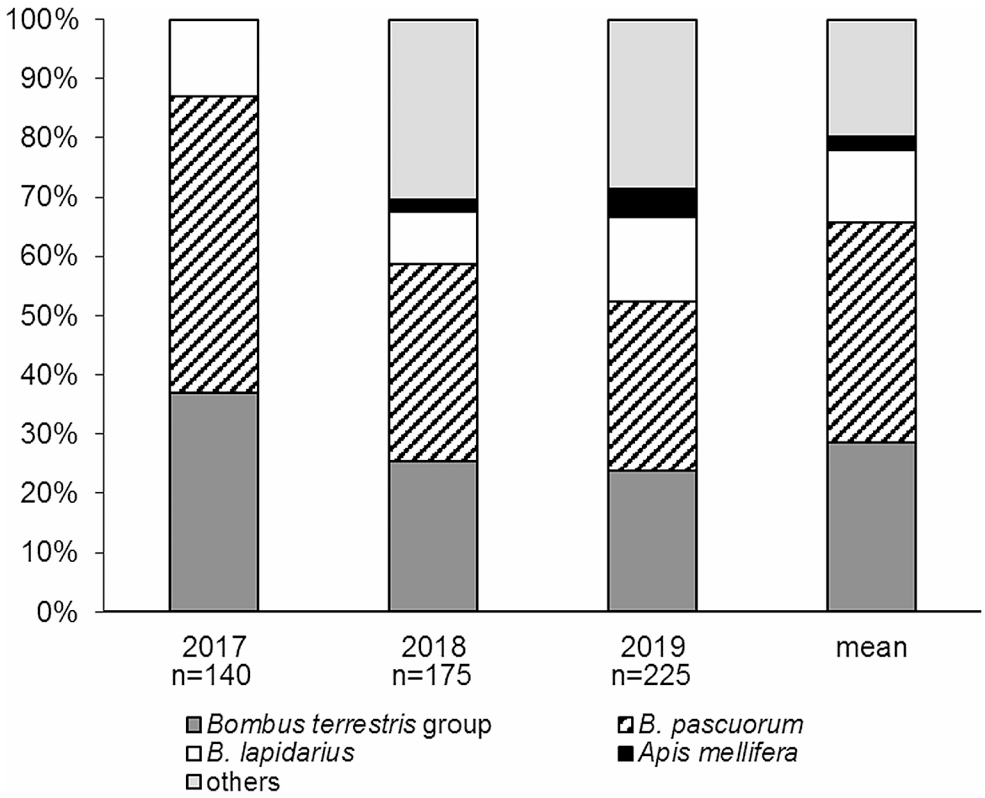

Fig. 5 Insect visitors spectrum in *S. carniolica* in 2017–2019 in Lublin, SE Poland

serve as a visual guide towards floral rewards. Moreover, the color-changing nectary disc may act as an indicator of the absence/presence of the floral nectar. The change in nectary disc color during flower development has also been observed in other Solanaceae (e.g., *Nicotiana* ssp.) and is thought to improve flower attractiveness to pollinators (e.g., Lunau et al. 2006; Nicolson and Thornburg 2007).

The surface of the interior part of the corolla bears numerous non-glandular trichomes. Such trichomes can also provide protection against biotic and abiotic stresses (Willmer 2011; Karabourniotis et al. 2020). According to Lipiński (2010), in the flowers of *Atropa belladonna* (Solanaceae) non-glandular trichomes limit access to nectar, especially for short-tongued insect visitors. In *S. carniolica*, non-glandular trichomes were found to accumulate tannins, alkaloids, and flavonoids, which indicates the integrated role of these structures in floral nectar protection against abiotic stresses, e.g., they can protect the corolla against water loss, UV radiation and temperature extremes. Moreover, they can be involved in defense against the herbivores and/or pathogenic agents derived from external sources (air, antagonist insect visitors). Chemical molecules detected in non-glandular trichomes display a wide range of biological activities, e.g., antifungal and antibacterial properties (Kaczmarek 2020 and literature cited therein). Additionally, the involvement of non-glandular trichomes against other than pathogen particles (e.g., sand, dust, dirt) has been indicated by Pozo et al. (2012).

Essential oils were also present in the cells of non-glandular trichomes localized on the corolla surface. Therefore, it is possible that these structures are engaged in fragrance secretion. We cannot neglect the possible functions of non-glandular trichomes as floral reward quality guides as suggested by diverse authors (e.g., Sulborska et al. 2014; Jachuła et al. 2018). Floral scent can originate from different structures, e.g., scent glands or osmophores (Antoń et al. 2012). In general, the potency and activity of essential oils are correlated with their chemical composition (Baratta et al. 1998). Moreover, the changes in the essential oils composition can be repellent or attractive to potential pollinators, i.e., may incur both a benefit (pollination) and costs (lack of pollination) (e.g., Kessler and Halitschke 2009; Novaković 2019; Robustelli della Cuna 2021). The nectary type and nectar traits (amount, sugar concentration, total sugar amount, nectar chemical composition) may be associated with the phylogenetic history (Nepi et al. 2009) or the pollination system (Noutsos et al. 2015). In *S. carniolica*, the nectary disc is located at the basal side of the ovary, as shown in the present study providing the first description of the nectary in this species. As reported by Phukela et al. (2021), the nectary positioned in the basal region of the ovary is characteristic for most Solanaceae species. It has been documented that a basal-positioned nectary ensures successful pollination (Kerchner et al. 2015). In Solanaceae species, the disc-type nectary predominates among actinomorphic flowers, which are mainly bee-pollinated (Phukela et al. 2021).

In this study, the *S. carniolica* flowers rewarded insect visitors with both nectar and pollen. Nectar secretion started in the bud stage and continued through the life-span (both female and male phases) of the flower as has been found in other species studies so far (e.g., Nepi and Stpiczyńska 2007; Stpiczyńska et al. 2014). However, nectar secretion peaked on the 2nd day of anthesis, i.e., at the moment of anther dehiscence and nectar was present till the end of anthesis. The changes in nectar production rate may have evolved to increase the chance of successful pollination, e.g., Antoń and Denisow (2014). Moreover, the nectar secretion pattern indicates that the nectar rewards availability to insect visitors may be connected with enhancement of male reproductive success in *S. carniolica.* A greater investment in male function is in line with the sexual selection theory in plants (e.g., Aizen and Basilio 1998; Carlson 2007). From the 3rd day, a decrease in the amount of nectar sugars was observed, indicating the beginning of the process of sugar resorption. An active floral nectar reabsorbtion process has been documented in many species (reviewed in Nepi and Stpiczyńska 2007). Usually, the process is observed near the end of anthesis and is regarded as an energy-saving strategy allowing the recovery of energy invested in nectar production (Roy et al. 2017; Pyke et al. 2020).

The amount of nectar in the flowers (1.58 mg per flower, on average) and the total amount of sugars (0.52 mg per flower, on average) were relatively low. Small amounts of nectar have also been reported in various solanaceous species, e.g., *Lycium* spp. flowers offered < 5 µg of nectar (Bernardello and Galleto 2005). In *Nicotiana* species, the nectar amount was positively correlated with the corolla length. Moreover, the nectar volume was associated with predominant pollinators and varied from 0.64 µg/flower in small moth-pollinated species to 17.18 µg/flower in hawkmoth-visited species (Kaczorowski et al. 2005). Similarly, the amount of total sugars obtained in *S. carniolica* was lower than the values documented in other Solanaceae species, e.g., *Datura ferox* 0.73–1.86 mg/flower, *Petunia axillaris* 0.9–1.6 mg/flower, or *Nicotiana glauca* 3.1–6.7 mg/flower (Galetto and Bernardello 1993; Torres et al. 2013).

The nectar sugars available in *Scopolia* flowers are also lower than what is produced in the flowers of co-flowering herbaceous species, e.g., *Lamium album* 1.31 mg/flower or *Taraxacum officinale* (2.13 mg/inflorescence unit) (Sulborska et al. 2014; Hicks et al. 2016). However, the value of nectar for pollinators is related not only to its quantity but also its quality, and every floral resource is important

in early spring because it will increase food diversity (e.g., Goulson et al. 2008; Timberlake et al. 2019). The flowers of *S. carniolica* exhibited a mean nectar concentration ranging from 26 to 42% during anthesis. In general, temporal variations in the nectar sugar concentration can be related to nectary characteristics and activity (secretion and reabsorption) (e.g., Corbet 2003; Nicolson and Thornburg 2007). Moreover, the nectar characteristics reveal association with the corolla depth (Phukela et al. 2021). Usually, flowers with deep corolla tubes produce more diluted nectar compared to short tubes or shallow ones (Corbet 1978; Kaczorowski et al. 2005). Likewise, biotic and abiotic factors can modify the nectar sugar concentration. The potential biotic factors that can exert an impact on the nectar sugar concentration include microorganisms or enzymes derived from pollinator hypopharyngeal glands (Heil 2011). Among abiotic factors, relative humidity and temperature can directly influence the nectar sugar concentration (Nicolson and Thornburg 2007; Denisow and Antoń 2012; Nepi et al. 2012). Variations in meteorological factors may partly explain both the day-to-day and year-to-year differences in the nectar sugar concentration noted in our study.

The chemical analyses of nectar carbohydrates revealed that the *S. carniolica* nectar contained only fructose and sucrose. Sucrose predominated over fructose, i.e., the nectar in this plant is sucrose-dominant (sucrose content 51–100%; S/(G + F) ratio > 1, sensu Baker and Baker 1983). In general, the nectar sugar composition can be associated with evolutionary pathways, phylogenetic origin, and/or adaptation to the pollination syndrome (Baker and Baker 1983; Abrahamczyk et al. 2017). Sucrose-dominant nectars were also detected in other species from the Solanaceae family, e.g., *Datura ferox* (Torres et al. 2013). However, hexose-rich nectars have also been identified among Solanaceae species. In the hexose fraction, glucose can even dominate, as in the nectar of species from the genus *Lycium* (Galetto et al. 1998). Generally, sucrose-dominant nectars are characteristic for tubular flowers with a deep corolla (Percival 1961; Corbet 1978; Perret et al. 2001; Nocentini et al. 2012). For example, similarly to our study species, sucrose-dominant nectar was identified in tubular flowers of *Oenothera* species with a 4–6 cm long hypanthium (Antoń et al. 2017). Due to the predominance of sucrose, the nectar has lower osmolality (sucrose-rich solutions) than hexose nectars (hexose-rich solutions) and therefore evaporates more rapidly (Nicolson and Thornburg 2007). Hence, flowers with deep corolla tubes offer better protection against evaporation to nectar with sucrose solutions. Moreover, species that flower in early spring benefit from sucrose nectars, as low temperatures (noted frequently at night and often during a day during *S. carniolica* flowering) may partly prevent evaporation (Goulson 1999; Jürgens 2004). However, it has to be underlined that due to the lack of complex study on nectar composition among Solanaceae species it is difficult to indicate unequivocally whether the variability is related to adaptation to pollinator preferences or are influenced by phylogenetic and physiological/biochemical properties of species. Explanation of this variability requires future extensive study.

Nectar carbohydrates composition and the S/(G + F) ratio were similar irrespective of the floral sexual phases. This feature together with corolla morphological traits (floral tube length, non-glandular trichomes inside the corolla) can restrict access to nectar for short-tongued pollinators. Consequently, a high level of sucrose might be indicative of specialized pollination systems (e.g., Torres & Galetto, 2002 and references therein).

We found that sugar concentrations varied considerably while the S/(G + F) ratio showed very little variation. This indicates that that reabsorption is the same for the two sugars present in *Scopolia* nectar. Baker and Baker (1983) and Antoń et al. (2017) have also previously reported relatively small variations in the ratio S/(G + F) in diverse plant species. Moreover, based on biochemical study, Tiedge and Lohaus (2018) suggested that stability in the sugars composition and proportion (sucrose/hexoses ratio) can be associated with the balanced expression and activity of invertases (i.e., sucrose-cleaving enzymes) over a flower life-span. On the contrary, temporal changes of sugar concentration in nectar are greater as they are related to many changeable factors (e.g., nectary activity and abiotic agents – discussed above in more details). Our study species can be classified among good pollen-producing plants as the *S. carniolica* flowers produce more pollen than co-flowering herbaceous *Lamium album* or *L. purpureum* (mean = 0.18 mg/flower and 0.03 mg/flower, respectively; Denisow and Bożek 2008), earlier flowering *Tussilago farfara* (mean = 1.29 mg/capitulum; Denisow 2011) or woody *Prunus spinosa* (mean = 0.23 mg/flower; Dmitruk et al. 2023).

The *Scopolia* flowers were mainly attractive to bumblebees. Other bees (*Andrena* sp. – mining bee, *Apis mellifera* - honey bee) were also observed but in small numbers. Given its early phenology and flowering during the scarcity of early season food sources, *S. carniolica* can provide the food resources for bumblebee queens, which start their nesting season and survive until other floral resources become available. During our field observations, visiting insects were not caught, so it is was not possible to determine whether the flowers were visited by bumblebee queens or workers. Nevertheless, the flowering time of the plant coincides with the date of the first flights of bumblebee queens and the start of nest establishing (Jachuła et al. 2022). Bumblebees use as much as possible of pollen-producing plants in spring, e.g., *Salix* sp., *Prunus* sp., due to the fact that pollen chemistry

is a major factor influencing the growth and development of their colonies (Moerman et al. 2016; Wood et al. 2022). Eeraerts et al. (2021) described that after mass flowering of early spring trees, there is a food gap leading to a significant decrease in brood cells formed by *Osmia bicornis* and *O. cornuta*, which may also translate to other wild pollinators.

Bumblebees are generally declining in Europe, so ensuring their food resources is one of the essential activities of protection of these species (Goulson et al. 2008; Lye et al. 2012). Therefore, the propagation of *S. carniolica* in gardens can support bumblebees. Moreover, the species is an interesting element in ornamental gardening and is recommended for shaded areas (Borgen and Guldahl 2011).

Future study should determine whether secondary metabolites (including toxic tropan alkaloids – hyoscyamine, atropine, scopolamine) that occur in vegetative tissues across the Solanaceae family, e.g., in *Scopolia* or *Nicotiana* species (Irwin et al. 2014; Nowak and Wolbis 2002) are found in nectar and/or pollen of *S. carniolica*. As reported by Cook et al. (2013) nectar and pollen secondary compounds can have diverse effects (neutral, positive, negative) on pollinators' behavior, performance, and survival.

Conclusions

The micromorphological and histochemical attributes of the flower of *Scopolia carniolica* were described for the first time. Numerous non-glandular and a few glandular trichomes are present in the corolla surface. In the non-glandular trichomes a range of metabolites were detected: lipids, acid lipids, tannins and alkaloids. Sucrose-rich nectar is secreted during both female and male phase of the flower's life. A combination of traits and cues (flowering phenology, flower morphology, olfactory signal metabolites, quantity and quality of floral rewards) are likely responsible for attraction of bumblebees. *Scopolia carniolica* should be therefore considered in bumblebee conservation schemes.

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Author contributions BD contributed to conception and methodology of the study. KT and JJ made field observations and laboratory studies. MD carried out the microscopic sectioning and observations. MD and KT performed histochemical tests. JJ and BD made statistical analyses. BD, KT, JJ and MD prepared the first draft of the manuscript. BD supervised the study. All authors contributed to manuscript revision, read, and approved the submitted version.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

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