Do honeybees promote or reduce the reproduction of two rare plants in Bulgaria?

Ekaterina Kozuharova

Department of Pharmacognosy, Faculty of Pharmacy, Medical University, 2 Dunav St., 1000 Sofia, Bulgaria, email: ina@pharmfac.acad.bg

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To the memory of Prof. L. Walter Macior

Abstract. Two rare plant species protected by the Biodiversity Law – *Astragalus dasyanthus* and *Haberlea rhodopensis* – were observed, so as to list their flower visitors. The behaviour of the honeybees in close vicinity was recorded simultaneously. The aim was to test how they affect the pollination of these two plant species, presuming that honeybees may have neutral to negative impact on the pollination of rare plants. The observations revealed that honeybees preferred other plants for foraging in the studied communities. Since honeybees do not actually use *A. dasyanthus* and *H. rhodopensis* as foraging resources, they neither promote nor reduce the reproduction of these rare plants. *Astragalus dasyanthus* was actively visited by bumblebees – mainly *Bombus hortorum* and seldom by other bumblebee species, but not by honeybees. Honeybees were abundant in the vicinity but they collected food from *Astragalus onobrychis, Dorycnium herbaceum*, *Onobrychis arenaria, Thymus sibthorpi,i* and some other plants. *Haberlea rhodopensis* practically received no visits, except for a single nectar-collecting *B. hortorum* queen. In this study site honeybees were also very active but they pollinated *Geranium macrorrhizum*.

Key words: *Astragalus dasyanthus*, bumblebees, *Haberlea rhodopensis*, honeybees, pollination

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Introduction

The question whether honeybees promote or reduce the reproduction of some particular rare plants was raised by the Society for Biological Beekeeping, Bulgaria. The main goal of the Society for Biological Beekeeping is to produce honey far away from the hazards and pollutants accumulated in the result of human activity. Such beekeeping requires setting the bee-gardens in habitats that often may shelter rare and endemic plants. The principle of biological beekeeping is to maintain a balance with the other components of the natural habitats. That is why several entomophilous plant species with conservation significance were listed as possible objects for monitoring, e.g. *Haberlea rhodopensis* Friv. (*Gesneriaceae*), *Astragalus dasyanthus* Pall. (*Fabaceae*), *Onosma rhodopea* Velen., *Alkanna primulifolia* Griseb. and *A. stribrnyi* Velen. (*Boraginaceae*), and *Campanula lanata* Friv. (*Campanulaceae*), with respect to their distribution and possibilities for building bee-gardens under the principles of biological bee keeping.

Astragalus dasyanthus is a rare species for the Bulgarian flora and is protected in Bulgaria by the Biodiversity Law (2002). Its conservation status assessed according to the IUCN criteria is Critically Endangered (CR) (unpubl.). The plant strikes long tap roots and propagation is mainly by seed. This plant occurs rather restrictedly elsewhere in South and East Europe, extending northwards to Hungary and Central Russia (Vulev 1976). We studied one of the few populations in Bulgaria, which is located in Mt Ruen, in the vicinity of Boboshevo.

Haberlea rhodopensis is a rare species for the flora of the Balkan Peninsula protected by the Biodiversity Law (2002). It is a Balkan palaeoendemic, a Tertiary relict and a species listed under the Bern Convention (1982). Its conservation status assessed according to the IUCN criteria is Least Concern (LC) (unpubl.). The plant propagates vegetatively and by seed (Vassilev 1984). Along with this, this amazing plant is capable of surviving dehydratation in antibiosis (hybernating, Ganchev 1950; Stanev 1973); it is recognized under Bulgarian law as a medicinal plant (Medicinal Plants Act 2000). Recently, its flowers have become popular in homeopathy and this may truly endanger it, owing to extensive collection of flowers and hence reduced seed set and generative maintenance of the populations.

Honeybees, which themselves are non-native pollinators on most continents, and which may harm native bees and other pollinators, are nonetheless critically important for crop pollination (Vaissiere & al. 1984; Vaissiere1991; Dimitrov & al. 1994; Kearns & al. 1998). Honeybee efficiency may be dubious for some legume seed crops (Richards 1996). Honeybees and bumblebees are polylectic pollinators and they visit plants proportionally to their abundance. The choice behavior of both honeybees and bumblebees is largely determined by the reward experience of bees at flowers. In experiments with controlled amount of nectar in artificial feeders, bees have made their choice according to the proportion of reward rather than by their color, but morphology matters. Similar observations have also been made in the field, among flower varieties differing in nectar content (Pankiew 1967; Giurfa 1991; Duffield & al. 1993; Greggers & Menzel 1993; Greggers & Mauelshagen 1997; Gumbert 2000). Honeybees and bumblebees demonstrate very high flower constancy – the honeybee is very constant, bumblebees less so (Brittain & Newton 1933; Butler 1949; Grant 1950; Bozilova & Ančev 1969; Free 1970a, b; Macior 1974a, b; Bozilova & Chan 1976; Heinrich 1976a, b; Heinrich & al. 1977; Petkova 1981, 1982, 1983; Bauer 1983; Ohguchi 1983; O'Neal & Waller 1984; Schmidt 1984; Bühlmann & al. 1987; Jato & al. 1994; MacKenzie 1994; Wilson & Stine 1996; Stenstrom & Bergman 1998; Comba & al. 1999; Kozuharova & Ančev 2001; Kozuharova 2004, 2006, etc.).

Honeybees are known to have neutral to negative impact on the pollination of rare plants (plants that have small or fragmented populations). In mixed plant communities they pay less attention to the plant species with few individuals and leave them to bumblebees and other insects (Karron 1987; Vakhrameeva & Dlussky 1994). Also, honeybees may visit sporadically rare species and take pollen from them but it is highly probable that they place it on the flowers of abundant species and even block the stigma with nonspecific pollen from cornucopians (Vakhrameeva & Dlussky 1994). Floral visitation by introduced honeybees can reduce indirectly male fitness, fruit and seed production of the tropical *Clusia arrudae* – a dioecious species pollinated by resin-collecting *Eufriesea nigrohirta*. Honeybees simply remove the pollen, leaving almost nothing to the legitimate pollinators, which explains why the frequency of *A. mellifera* male flowers is negatively correlated with the number of seeds produced by female flowers (do Carmo & al. 2004).

In any one area with a variety of concurrently blooming plants bumblebees have apparent species preferences. Superimposed on these species preferences, individuals have primary foraging specialities (majors) and secondary specialities (minors) and this allows the bees to keep track of changing resources (Heinrich 1976a, b, 1979a). Bumblebee choice is often related to their proboscis length and body size (Pouvreau 1984; Brian 1951, 1957; Teräs 1976, 1985), and the flower morphology (Laverty 1994). Bumblebee species with long glossae have access to nectar in a greater variety of flowers than those with short glossae and they tend to feed from a larger number of plant species (Harder 1982). Bumblebees generally prefer flowers with corolla lengths in relation to their proboscis lengths but additionally they have different habitat preferences, emergence times, nest sites, colony development strategies that all indirectly affect flower choices (Teräs 1985). Assessed were the effect of plant density and the relative floral morphological complexity of plant species on the behaviour of their bumblebee pollinators (*Bombus pascuorum, B. terrestris and B. hortorum*). It appeared that rare plants had actually an advantage in terms of the number of bees attracted per plant. However, they were at a disadvantage in terms of pollen wastage, because foragers more often went to a flower of another species after visiting a rare plant. The behaviour of bees on each plant species was further affected by the plant's floral complexity and the identity of the other species in the array. The three bumblebee species were markedly different in their foraging behaviour and in their responses to varying floral density and complexity. Each species preferred some particular flower species (Stout & al. 1998a).

The aim of this study is to test the role of the honeybees in the process of pollination of *A. dasyanthus* and *H. rhodopensis* and further to observe and list other flower visitors as possible pollinators.

Material and methods

Plant species and floral characters

Astragalus dasyanthus is a perennial plant with massive rhizomes, tall stems (up to 30 cm) and long feathery leaves, with numerous, odd leaflets. There are several flowering stems and each bears several flower heads. Numerous pale-yellow flowers about 2 cm long are clustered densely.

Haberlea rhodopensis is a perennial plant with a leaf rosette, one to numerous flowering stems, each bearing single or few gullet white and purple flowers, about 1.5-3.5 cm long and clustered in small umbels with bracts.

Measured were the characters of single flowers that restrict access to the nectar of each plant species (calyx tube depth for the flowers *A. dasyanthus*, $n = 20$; corolla tube length and corolla tube width for the flowers of *H. rhodopensis*, $n = 20$).

Study sites

The field investigations were conducted in one of the few Bulgarian populations of *Astragalus dasyanthus*, near the town of Boboshevo, Mt Ruen (WGS84 42°09'08.6''N and 22°59'28.6''E, at 524 m, Plate I, Fig. 1) in June – early July 1995, 2007 and 2008. The population is of mosaic type, localized on a small stretch of just one slope, covering a territory of about 54 000 m^2. The habitat could be described as forest-steppe. *Haberlea rhodopensis* was studied in early July 2008, in the vicinity of Trigrad village, in the Rhodopes (WGS84 41°36'40.5''N and 24°22'42.1''E, at 1276 m). The population in the area of Trigrad is scattered in several patches (about 50 m^2 to 200 m^2 each and 100 to 1000 m apart). This spatial structure is in accordance with the habitat specifics of *H. rhodopensis*: it is a hasmophyte, with preference for shady and mossy vertical rocks (Plate II, Fig. 1). The habitats of both plant species are rich in flora and fauna and are chosen for the NATURA 2000 areas (Abadjiev & Beshkov 2007).

Activity and behaviour of the bees

The bees visiting the target plant species were observed by a site-transect method (Dafni 1992). Observations on *A. dasyanthus* took a total of 330 minutes within six days (two days in each 1995, 2007 and 2008) and on *H. rhodopensis* – 290 minutes within two days in 2008. The type of reward (pollen and/or nectar) was recorded as well. Pictures were taken with Olympus E 500 and Zuiko Digital 14-45 mm f3.5-5.6. Mean activity of the bees was calculated as a quotient of the number of pollinators recorded and the minutes of observation multiplied by 60 minutes. Eleven of the bumblebees observed in the flowers of *A. dasyanthus* were collected and immobilized in a tube in order to extract their pollen load. Pollen contamination was avoided by catching each bumblebee separately. Pollen identification and counting (at least 1000 pollen grains after Louveaux & al. 1978) was conducted under the Amplival Carl Zeiss Jena light microscope, at magnification ×160 and ×640.

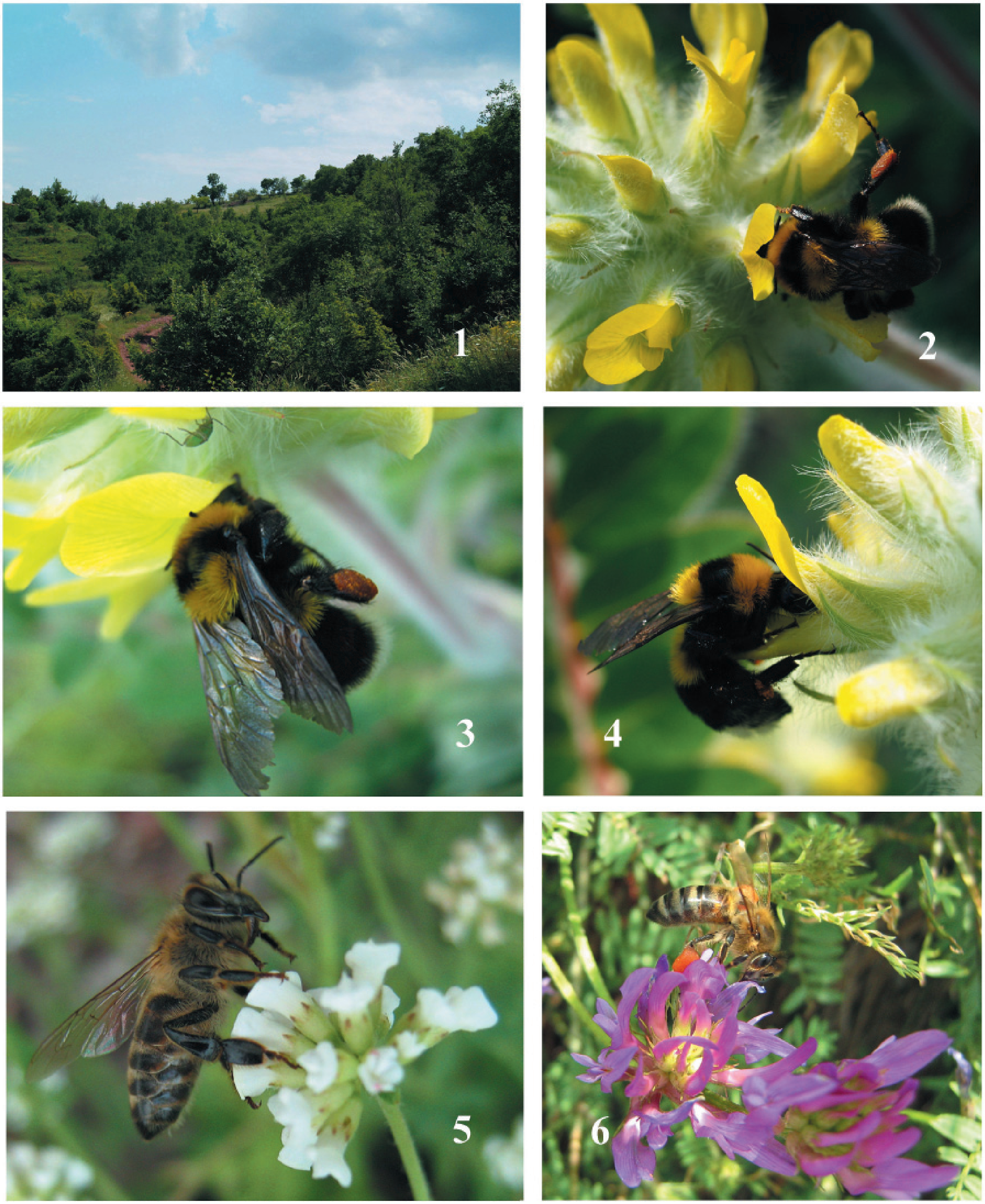
Sympatric and simultaneously flowering entomophylous plant species (potential pollinator competitors) in the neighborhood of *A. dasyanthus* and *H. rhodopensis* and their insect visitors were recorded too. Special emphasis was laid on the honeybees. The plants are listed in abundance order estimated in the very close vicinity of the studied plants (Table 1).

Results and discussion

Astragalus dasyanthus

The flower is full of nectar: a big drop of it was observed with a naked eye on the dorsal part of the ovary of each of the studied flowers ($n = 1\ 0$). It is of the "flag" type (sexual organs are found in the lower part, pollen is deposited on the abdominal side of the insect, sternotribic pollination, Faegri & van der Pijl 1971). The flowers were actively visited by bumblebees – mainly *B. hortorum* L. and seldom by other species, sporadically visited by carpenter bees – *Xylocopa* sp., but no honeybees were recorded visiting them (Table 1). In 1995 and 2008, the visits were dominated by *B. hortorum*. The main visitors in 2007 were of *B. terrestris* L. (Table 1). Similar variation in the species composition of bumblebees through the years was observed in the population of *A. alopecurus* (Kozuharova & Firmage 2006). Annual peculiarities of bumblebee colonization and competition are the possible reasons for this phenomenon. Col-

Plate I



1, *Astragalus dasyanthus*: the habitat; **2-4**, *Bombus hortorum* workers collecting nectar from *A. dasyanthus*; **5,** *Apis mellifera* workers collecting nectar from *Dorycnium herbaceum*; **6**, *A. mellifera* workers collecting food from *A. onobrychis*.

Plate II

1, *Habrelea rhodopensis*: the habitat; **2**, Flowers of *H. rhodopensis*; **3,** Honey bee landing on *Geranium macrorrhizum*; **4,** Calyx and ovary with the nectar ring; **5,** Corolla of *H. rhodopensis*: **a** – corolla tube depth; **b** - corolla tube width.

Table 1. Foraging plants and mean activity of the bees (calculated as a quotient of the number of pollinators recorded and the minutes of observation multiplied by 60 minutes).

Rare plants and activity of their bee visitors	**Abundant entomophylous plants in the neighborhood**	**Activity of the bees and other insects visiting the respective abundant entomophylous plant species**
Astragalus dasyanthus	**1995, 2007 and 2008 - Boboshevo**	**1995, 2007 and 2008 - Boboshevo**
	Doricnium herbaceum	*Apis mellifera* **23.5**
1995	*Astragalus onobrychis*	*A. mellifera* **18.1**
Activity 5.4 – 10.2	*Thymus sibthorpii*	*A. mellifera* **10.5** *Halictus* sp. **not measured**
Bombus hortorum	*Onobrychis arenaria*	*A. mellifera,* L. **6.5** *Halictus* sp. **not measured**
W ($n = 20$), M($n = 3$)	*Orlaja grandiflora*	*Syrphydae* **not measured**
B. soroensis	*Digitalis lanata*	
W ($n = 1$)	*Alyssum murale*	
Xylocopa sp.	*Teucrium chamaedrys*	
F ($n = 1$)	*T. pollium*	
2007	*Onosma aucherana*	
Activity 15 – 17.4	*Anthemis tinctoria*	
B. terrestris	*Hypericum umbellatum*	
W ($n = 5$)	*Dianthus pinifolius*	
B. hortorum	*Medicago falcata*	
W/Q ($n = 2$)	*Potentilla sulphurea*	
B. lapidarius	*Centaurea rhenana*	
W ($n = 2$)		
Xylocopa sp.		
F ($n = 1$)		
2008		
Activity 13.1 – 21.0		
B. hortorum		
W ($n = 37$)		
Haberlea rhodopensis	**2008 – Trigrad**	**2008 - Trigrad**
	Geranium macrorrhizum	*Apis mellifera* **7.5–31.1**
2008		*Bombus agrorum* **1.5**
Activity – 0.21		*B. pratorum* **0.5**
B. terrestris	*Scrophularia canina*	*B. pratorum* **4.5**
Q ($n = 1$)		*Vespula* sp. **1.1**
Nectar collecting queen		

onization dynamics depends on many factors (Pittioni 1939; Heinrich & Raven 1972; Heinrich 1975, 1979b; Bowers 1985). The bumblebees, as a rule, collected nectar in the flowers of *A. dasyanthus*. Although some of them cannot reach the very bottom of the calyx tube (Table 2), they obviously can reach the nectar on the ovary. The bumblebees transferred the pollen sternotribically (front lower part of the thorax, and lower part of the head and tongue (Plate I, Fig. 4). Most workers had full pollen baskets as they cleaned their face and transferred the pollen grains into the baskets, while flying from flower to flower (Plate I, Figs 2–4). Only few of all observed workers collected pollen briefly (2-3 movements with front legs only), directly from the anthers, and this happened after the nectar extraction in the same flower just before they left it. Macior (1969, 1973, 1982, 1984) noted the use of front legs to scrape pollen from the anthers of several *Pedicularis* species (*Scrophulariaceae* – “gullet” flowers, sexual organs are restricted to the functionally upper side, pollen is deposited on the insect back and upper part of the head, nototribic pollination, Macior 1969, 1973, 1982, 1984). Kwak (1977) did not observe bumblebees to scrape pollen from the anthers in her study of five *Rhinanthoideae* species (*Rhinanthus*, *Pedicularis* and *Melampyrum,* the same family with the “gullet” flowers), where bumblebees were sternotribic pollinators: vibrating pollen from the anthers upside down. She noted that bumblebees groomed pollen from the venter during the flight after visiting one or several flowers. Furthermore, she reported visits by honeybees in these “bumblebee” flowers for pollen, while they seldom collected nectar and often through holes perforated earlier by bumblebee nectar thieves (Kwak 1977, 1980).

On a single foraging trip bumblebees visited up to 25 flower heads of *A. dasyanthus* (5-6 flowers per head)

Table 2. ▹ **Flower morphology and tongue lengths**
*Pekkarinen 1979; ** Puvreau 1984; ***Barbola & al. **2006; **** Goulson & Stout 2001.**

species/characters	**Bumblebees**	**Tongue length**
Astragalus dasyanthus Restriction for access to the nectar Calyx tube measurements: (*n* = 20) Calyx tube depth 9.4 ± 0.76 mm Calyx tube width 5.1 ± 0.58 mm	*Bombus hortorum*	Q 14.6 ± 0.57 mm*; 18–21.2 mm ** W 12.42 ± 0.57 mm*; 12.8–16.1 mm **
	Xylocopa sp.	8,8 mm***
	B. lapidarius	W 6.96 ± 0.68 mm *; 8.4–10.6 mm **
	B. soroensis	W 6.77 ± 0. 54 mm *; 8.0–9.22 mm **
	B. terrestris	W 7.85 ± 0.57 mm*; 7.6–8.8 mm **
	Apis mellifera	W 6.6 mm
Haberlea rhodopensis Restriction for access to the nectar Corolla tube measurements (*n* = 20): Corolla tube depth 15.0 ± 1.58 mm Corolla tube width 6.8 ± 0.53 mm		
	B. hortorum	Q 14.6 ± 0.57 mm*; 18–21.2 mm ****
	A. mellifera	W 6.6 mm ****

on 15 flowering stems. There were available up to 15 flowers on a head, each flowering stem has usually 6 (4-7) flowering heads and each plant has one to several (4-5) flowering stems. Bumblebees moved first horizontally and then upwards. The behavior of these *B. hortorum* individuals was very similar to that of a colony of the same species in the flowers of *A. alopecurus* in the Rhodopes (Kozuharova & Firmage 2007). Occasionally, bumblebees used to hover before taking a decision for landing. This behaviour is well explained in a number of studies: bees are known to discriminate between flowers and avoid those that are less rewarding, which confers great advantages; in addition to visual cues to floral rewards they use the presence of hydrocarbons left behind by previous insect visitors and thus avoid flowers that have been recently visited (Morse 1986; Schmitt & Bertsch 1990; Goulson & al. 1998, 2000, 2001; Stout & al. 1998b; Goulson & Stout 2001; Stout & Goulson 2002; Goulson 2003; Goulson & al. 2007). The bumblebees probed an average of several flowers per head.

The extremely high flower constancy of bumblebee workers observed in the field correlated with the high percentage of *Astragalus*-type pollen found in their pollen baskets (between 98.1 % and 100 %, *n* = 11). The highest recorded activity (Table 1) was at the end of the flowering period of *A. dasyanthus,* on a very hot day (30 °C), when the bumblebees “desperately” collected nectar and the baskets were usually empty. Possibly, the colony abounded in workers of several generations at that later time of the season. It was also possible that the heat stimulated nectar secretion, or that sugar concentration was higher, but this calls for a research to test this assumption. Another possible explanation of the observed behavior was that the bumblebees were dehydrated.

Honeybees were abundant in the vicinity of *A. dasyanthus* but they collected food from *A. onobrychis, Dorycnium herbaceum*, *Onobrychis arenaria*, *Thymus sibthorpii,* and some other plants (Table 1; Plate I, Figs 5–6).

Haberlea rhodopensis

The flower has a bright-orange nectar ring at the bottom (Plate II, Fig. 2). For comparison, its closest relative *Jankea heldreichii* (another of the three members of the Gesneriaceae family in the Balkans, also a Tertiary paleoendemic) has flowers without nectar (Vokou & al. 1990). *Habrelea rhodopensis* and *J. heldreichii* are not only taxonomically close. They have very similar habitat preferences: shady limestone rocks (Plate II, Fig. 1). And their resemblance continues with abundant co-occurring plants – for both of them it is *Geranium macrorrhizum* (Table 1, Vokou & al. 1990). *Haberlea rhodopensis* received practically no visits, except for a single nectar-collecting *B. hortorum* queen. Although further experiments to test the breeding system of *H. rhodopensis* are necessary, the presence of herkogamy (anthers fused beneath the stigma, Plate II, Fig. 5) and not fertilized wilted flowers indicate the need of insect vectors for pollination. Flower size should correspond to the pollinators. In this case the depth (15.0 ± 1.58 mm) and width (6.8 ± 0.53 mm) of the corolla tube, as well as the long hairs on the lower lip restrict the nectar access to long-tongued insects (Table 2; Plate II, Fig. 5). Most probably, cross-pollination of this spring-early summer flowering relict plant is accomplished by nectar-collecting bumblebee queens with long proboscises. Such adaptation for pollination by nectar-collecting queens is described for members of the related family *Scrophulariaceae*: a number of *Pedicularis* species (Macior 1982). Moths cannot be excluded from possible pollinators, as the flowers do not close at night. Predominantly bumblebee queens visited *J. heldreichii*. However, they collected pollen, as the flowers had no nectar (Vokou & al. 1990). Visitors were scarce in the flowers of both gesneriads (Table 1, Vokou & al. 1990). The corolla of only one of all observed *H. rhodopensis* flowers (*n* = 40) had a hole – an evidence of nectar robbing. Of the five species of bumblebees observed foraging on *Linaria vulgaris (Scrophulariaceae*, a related family to *H. rhodopensis*), the short-tongued *Bombus lapidaries, B. terrestris* and *B. lucorum* used to rob nectar, whilst longer-tongued *B. hortorum* and *B. pascuorum* behaved as legitimate pollinators (Stout & al. 2000).

Honeybees were very active in the vicinity of *H. rhodopensis*. However, flower morphology of *H. rhodopensis* was not convenient for them: the corolla tube was twice as long as their tongue, and narrow enough so that they could not enter (Table 2). The honeybees pollinated *G. macrorrhizum* (Table 1). They collected nectar from its flowers (Plates II, III). This cranesbill was quite abundant around one patch of *H. rhodopensis*, whilst in the other sites the ratios of the plant species were similar (1.5 to 2 times in favour of *G. macrorrhizum)*. The highest recorded activity was in the big patch (covering about 3000 m^2) of this plant species. Honeybees are common visitors of *G. macrorrhizum* elsewhere in Bulgaria, predominantly for nectar, along with several bumblebee species (Kozuharova 2002). For comparison, honeybees accounted for one-third of the flower visitors of *G. pratense,* but were scarce in the flowers of *G. palustre.* The latter had a very flexible pedunculus and was pollinated by smaller and lighter insects – various flies (Dlussky & al. 2000). Cross-pollination by anthophilous flies (*Diptera*) was observed in *G. sylvaticum,* while *G. phaeum* with its pendulous flowers adapted for "buzz" pollination was visited for pollen by bumblebees: *B. agrorum* (Kozuharova 2002).

The other co-flowering species with *H. rhodopensis* was *Scrophularia canina* L. and it was visited by workers *B. pratorum* and *Vespul*a sp. (Table 1).

Conclusion

Since honeybees did not actually use *Astragalus dasyanthus* and *Haberlea rhodopensis* as foraging resources, because they have preferred other plants in the studied communities, the conclusion is that honeybees have not direct influence on the pollination of these plant species. In other words, the observations presented here indicate that honeybees play a neutral role in the pollination of the two tested rare plant species. The expected negative impact observed in other cases – pollen taken by honeybees from the rare plant and placed on flowers of an abundant species and even blocking of its stigma with nonspecific pollen from other plants (Vakhrameeva & Dlussky 1994) – was not confirmed for *A. dasyanthus* and *H. rhodopensis*.

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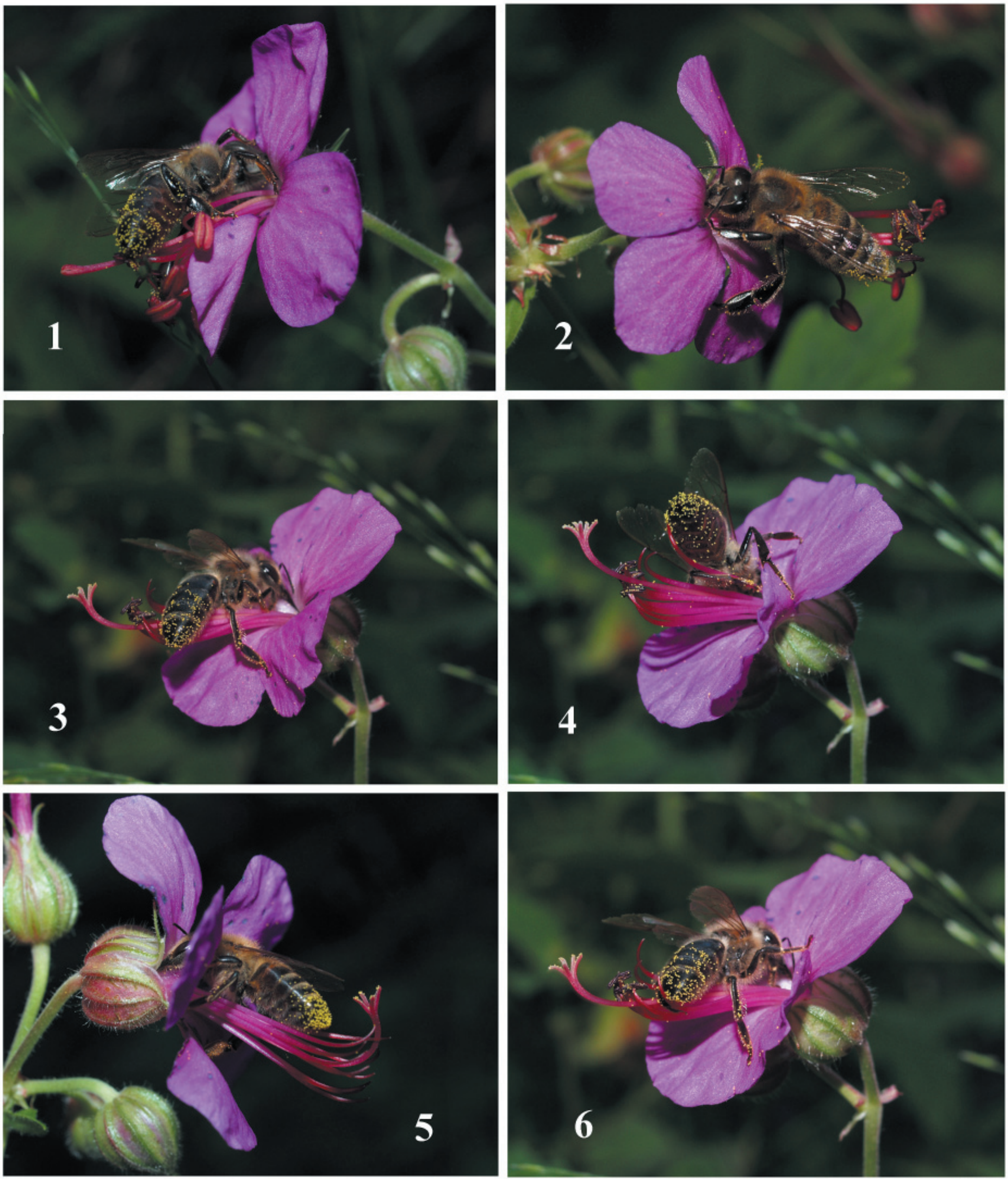
Plate III



Honeybees collect nectar in the flowers of *Geranium macrorrhizum* and leave small injuries on the petals with their claws (the bluish spots).

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