

Original Article

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Bumblebees and pollination of endemic *Onobrychis pindicola* (Fabaceae) in the subalpine habitats of Pirin Mts.

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Abstract:

Kozuharova, E.: Bumblebees and pollination of endemic *Onobrychis pindicola* (Fabaceae) in the subalpine habitats of Pirin Mts.. *Biologica Nyssana*, 9 (2). December, 2018: 89-101.

Onobrychis pindicola subsp. *urumovii* Degen & Dren. is an endemic with restricted distribution on Pirin Mts. and Slavjanka Mts. (Balkan Peninsula, SW Bulgaria). It is obligatorily dependent upon insect vectors for pollen transport, being a self-incompatible plant. The aim of this study was: 1) to identify which bumblebees pollinate *Onobrychis pindicola*, 2) to identify which plants compete for these pollinators with *O. pindicola*, 3) to use pollen load analysis to assess bumblebee flower constancy and spatial foraging patterns of bumblebee pollinators of *O. pindicola*. The nectar is accessible to a wide spectrum of bees including short tongued bumblebees and honey bees. *Onobrychis pindicola* is pollinated most actively by *Bombus pyrenaicus*. Other bumblebees visited the flowers more or less actively. These were *B. lapidarius*, *B. terrestris* and *B. pratorum*. The bumblebees tended to utilize plant resources in guilds and follow particular plants for foraging. Observed in the field high flower constancy was confirmed by the pollen analysis.

Key words: pollen loads, flower constancy, bumblebees, foraging

Apstrakt:

Kozuharova, E.: Bumbari i polinacija endemične vrste *Onobrychis pindicola* (Fabaceae) na subalpskim staništima planine Pirin. *Biologica Nyssana*, 9 (2). Decembar, 2018: 89-101.

Onobrychis pindicola subsp. *urumovii* Degen & Dren. je endemit sa rasprostranjenjem ograničenim na Pirin i Slavjanku (Balkansko poluostrvo, JZ Bugarska). Ona je obligatno zavisna od insekata vektora za transport polena jer je samo-nekompatibilna vrsta. Cilj ovog rada bio je da: 1) identifikuje koji bumbari vrše polinaciju *Onobrychis pindicola*, 2) da identifikuju koje su biljke u kompeticiji sa *O. pindicola* za ove polinatore, 3) da se iskoristi analiza polenskog tovara kako bi se utvrdila konstantnost biljnih vrsta i obrasci hranjenja na nivou vrste bumbara oprašivača *O. pindicola*. Nektar je dostupan širokom spektru vrsta bumbara uključujući one sa kratkim jezikom kao i pčele. *Onobrychis pindicola* je najčešće oprašivana od strane *Bombus pyrenaicus*. Ostale vrste bumbara bile su manje ili više aktivne u posećivanju cveta ove vrste. To su bile vrste *B. lapidarius*, *B. terrestris* i *B. pratorum*. Bumbari su koristili biljne resurse u gildama i pratili određene biljke tokom sabiranja polena. Tokom terenskih istraživanja utvrđena je visoka cvetna stalnost, što je potvrđeno analizom polena.

Ključne reči: polenski tovar, cvetna stalnost, bumbari, sabiranje polena

Introduction

Onobrychis pindicola subsp. *urumovii* Degen & Dren. (Fabaceae) is an endemic with very restricted distribution (**Fig. 1**) on just two mountains on the Balkan Peninsula, SW Bulgaria, namely Pirin Mts. and Slavjanka Mts. (Velchev, 1992; Euro+Med PlantBase, 2011). It is a calciphilous perennial plant that forms dense tufts and has an almost vertical reddish-brown rhizome. Its stems are short, or it may lack stems altogether. Its leaves are pinnately compound and normally bear four to seven pairs of lanceolate, hairy leaflets and a similar terminal leaflet, and its numerous purple flowers are borne on dense racemes. The legume is round and dentate (Kozuharov, 1976). *Onobrychis pindicola* subsp. *urumovii* falls in the category of abundant species even though it is restricted in distribution. Therefore it was assessed, according to the IUCN criteria (Petrova & Vladimirov, 2008), as “least concern”. At the same time *O. pindicola* subsp. *urumovii* is an element in several habitats which possess conservation significance – Natura 2000 habitats: 6170 - alpine and subalpine calcareous grasslands, 8120 calcshist screes, 4060 dominated by *Juniperus*

woods of *Pinus peuce* and *Pinus heldreichii* (Roussakova, 2015).

We previously studied the breeding systems of *Onobrychis pindicola* growing on Pirin marbles and the plants were found to be self-incompatible and dependent upon insect vectors for pollen transport (Kozuharova, 1999; Kozuharova & Richards, 2016).

The aim of this study was to: 1) identify which bumblebees pollinate *Onobrychis pindicola*, 2) to identify which plants compete for these pollinators with *O. pindicola*, 3) to use pollen load analysis to assess bumblebee flower constancy and spatial foraging patterns of bumblebee pollinators of *O. pindicola*.

Material and methods

Study sites

The field observations were conducted in the marbleized karst regions of North Pirin Mts., namely the main watershed of North Pirin Mts. (**Fig. 1**). Seven study sites of 40 m² were chosen. Study sites were grouped in the following habitats: Natura 2000 habitat 6170 – study sites 10, 11 and 15 (subalpine calcareous grasslands), study sites 3 and 4 (alpine calcareous grasslands); Natura 2000 habitat 4070 – study site 20 (dominated by *Pinus mugo*); Natura 2000 habitat 95A0 – study site 12 (woods of *Pinus heldreichii*). The altitude ranges between 1850 – 2410 m a.s.l.

The period of investigations was during the summers of 1995, 1996, 2001, 2002, 2005, 2014 and 2015.

Access to the nectar

A minimum number of two flowers per plant and a minimum of five plants per location were used to measure the depth of the calyx tube in order to evaluate the restriction for access to the nectar.

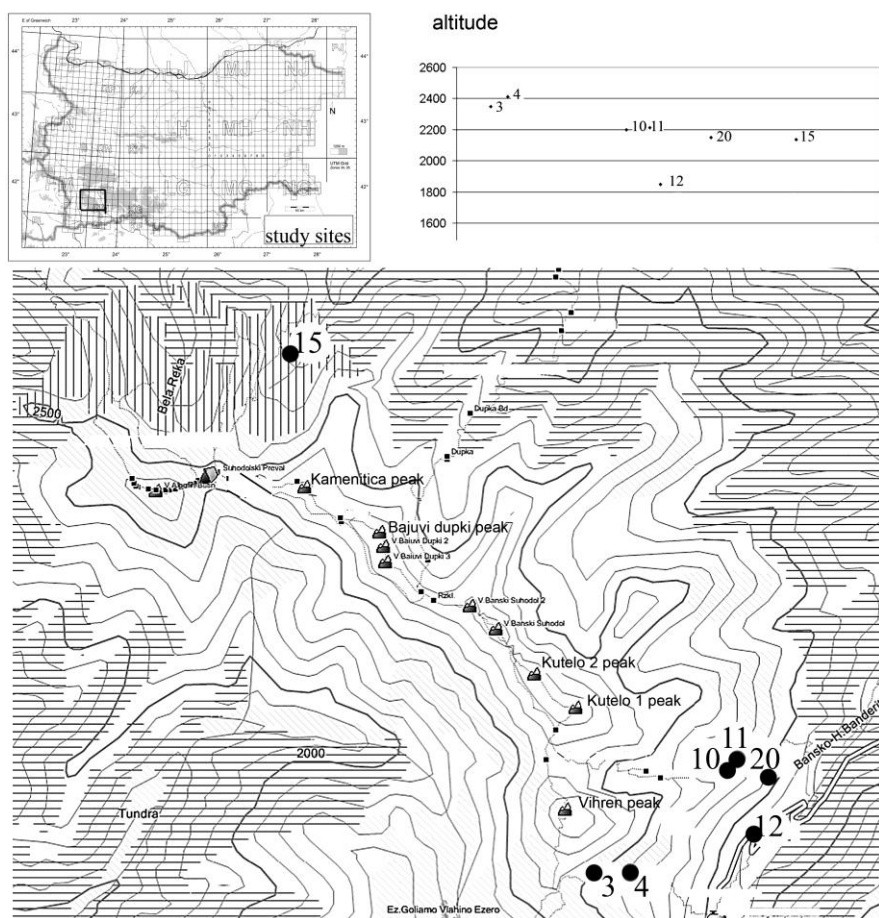


Fig. 1. Map of the study area

sibirica, 4070 dominated by *Pinus mugo*, 95A0 –

Table 1. Visitation index of bumblebees pollinators of *Onobrychis pindicola* and sympatric simultaneously flowering plants [average value for the whole period of observations]. Abundance according to Drude (1913) scale: Soc. (sociales), Cop.3 (copiosae3), Cop.2 (copiosae2), Cop.1 (copiosae1), Sp. (sparsae), Sol. (solitariae)

	<i>Bombus pyrenaeus</i>	<i>Bombus lapidarius</i>	<i>Bombus terrestris</i>	<i>Bombus pratorum</i>	<i>Apis mellifera</i>
Study sites 3 & 4					
Available flowers of a <i>Onobrychis pindicola</i> 6 600					
<i>Onobrychis pindicola</i> Cop.3	18.9	9.0	3.0		
<i>Gentiana verna</i> Cop.1				3.0	
<i>Anthyllis vulneraria</i> Cop.3					
<i>Helianthemum nummularium</i>					
<i>Linum capitatum</i> Cop.3					
<i>Thymus</i> sp. Cop.1	1.5				
<i>Acynus alpinus</i> Cop.3				0.6	
<i>Teucrium montanum</i> Cop.1	3.6	1.2			
<i>Dianthus cruentus</i> Cop.3					
<i>Campanula velebica</i> Sol					
<i>Oxytropis campestris</i> Cop.3	2.0	8.6			
Study sites 10 & 11					
Available flowers of <i>Onobrychis pindicola</i> 20 000 000					
<i>Onobrychis pindicola</i> Cop.3	31.4	2.6	1.5	2.2	0.6
<i>Carduus candicans</i> Cop.3		10.8			
<i>Centaurea rhenana</i> Cop.2		6.0			
<i>Clinopodium vulgare</i> Cop.2					
<i>Dianthus petreus</i> Cop.1					
<i>Galium gr. molugo</i> Cop.2					
<i>Acynus alpinus</i> Cop.2		1.8			
<i>Trifolium medium</i> Cop.1					
<i>Anthyllis vulneraria</i> Cop.3		8.0			
Study site 20					
Available flowers of <i>Onobrychis pindicola</i> 1 000					
<i>Onobrychis pindicola</i> Cop.3	6.3	3.6	0.9	2.4	3.0
<i>Linum capitatum</i> Cop.3					
<i>Scorconera rosea</i> Sol					
<i>Polygala major</i> Cop.2					
<i>Helianthemum nummularium</i> Cop.1					
<i>Rhodax canum</i> Cop.1	1.2				
Study site 12					
Available flowers of <i>Onobrychis pindicola</i> 1 000					
<i>Onobrychis pindicola</i> Cop.3	7.4	0.6	0.3	1.9	0.1
<i>Linum capitatum</i> Cop.3					
<i>Polygala major</i> Cop.2					
<i>Geranium sylvaticum</i> L. Cop.2					
<i>Rubus idaeus</i> Cop.2					
<i>Helianthemum nummularium</i> Cop.1				0.1	
<i>Rhodax canum</i> Cop.1	0.1			5.4	
<i>Centaurea rhenana</i> Sp					
<i>Scorconera rosea</i> Sol					
Study site 15					
Available flowers of <i>Onobrychis pindicola</i> 5 000					
<i>Onobrychis pindicola</i> Cop.3	14.4	24.0		5.4	5.4
<i>Hypericum tetrapterum</i> Cop.3		9.0		12	3
<i>Scutellaria alpina</i> Cop.3					
<i>Knautia midzurensis</i> Cop.2	13				
<i>Acinos alpinus</i> Cop.2					
<i>Thymus</i> sp. Cop.1	4.2				
<i>Carduus candicans</i> Cop.1	15				
<i>Dianthus cruentus</i> Cop.1					

Table 2. Functional flower/blossom morphology (according to Faegy, van der Pijl 1971) of plant sympatric species blooming simultaneously with *O. pindicola*; Abundance according to Drude (1913) scale: Soc. (sociales), Cop.3 (copiosae3), Cop.2 (copiosae2), Cop.1 (copiosae1), Sp. (sparsae), Sol. (solitariae)

Functional flower morphology	magenta	cyan	yellow	white
Dish/bowl	<i>Geranium sylvaticum</i> L. Cop.2		<i>Helianthemum nummularium</i> (L.) Mill. Cop.3 <i>Rhodax canus</i> (L.) Fuss. Cop.3 <i>Alyssum cuneifolium</i> Ten. 5 Cop.1	<i>Galium gr. molugo</i> Cop.1 <i>Rubus idaeus</i> L. Cop.2
Dish/bowl+ funnel/tube	<i>Centaurea triumphetti</i> All. 1 Sp. <i>Scorzonera purpurea</i> L.2 Sol. <i>Carduus</i> sp. Sol. <i>Centaurea rhenana</i> Boreau Sp. <i>Scabiosa lucida</i> L. Sol.	<i>Jasione laevis</i> Lam. 3 Cop.1	<i>Hieratium naegelianum</i> Panc. Sp. <i>Hieratium hoppeanum</i> Schult. Sp.	<i>Achillea ageratifolia</i> (Sm.) Boiss. Sp.
Funnel shallow	<i>Armeria alpina</i> Willd. Sol.		<i>Linum capitatum</i> Kit. ex Shult Cop.3	<i>Cerastium alpinum</i> L. Sp.
Funnel deep	<i>Dianthus cruentus</i> Grsb. Sp.	<i>Gentiana verna</i> L. Sp.		<i>Dianthus petraeus</i> W. et K. Sp.
Bell		<i>Campanula velebitica</i> Borbas. Cop.1		
Flag	<i>Onobrychis pindicola</i> Hausskn.4 Cop.3 <i>Trifolium medium</i> L. Sol.		<i>Anthyllis vulneraria</i> L. (s.l.) Cop.3 <i>Chamaecytisus absinthioides</i> (Janka) Kuzm.Cop2 <i>Oxytropis campestris</i> (L.) DC. Cop.3	
Gullet	<i>Polygala major</i> Jacq. Cop.2 <i>Clinopodium vulgare</i> L. Sol. <i>Acinos alpinus</i> (L.) Moench Cop.2 <i>Scutellaria alpina</i> L. Cop.3 <i>Stachys alpina</i> L. Sp. <i>Thymus perinicus</i> (Vel.) Jalas Cop.2 <i>Thymus moesiacus</i> Vel. Cop.2	<i>Ajuga genevensis</i> L. Sp.	<i>Teucrium montanum</i> L. Cop.1	

Pollinators – species composition, activity and foraging behaviour

Total observations time was 720 minutes. The bees were observed using a site-transect method according to Dafni (1992). Bumblebees were identified in the field according to Pittioni (1938; 1939), Edwards and Jenner (2005), and Gogala (2015). Their behaviour was recorded.

Evaluation of foraging plants availability

The approximate abundance evaluation of the plant species was conducted using the Drude scale, as

follows: Soc. (sociales), Cop.3 (copiosae3), Cop.2 (copiosae2), Cop.1 (copiosae1), Sp. (sparsae), Sol. (solitariae) (Drude 1913, Dimeyeva et al. 2016). We chose to use the Drude scale, as this approach has particular importance for evaluation of those plant species that grow in close vicinity to *Onobrychis pindicola* and bloom at the same time as it. The approximate number of fully open flowers of *Onobrychis pindicola* available to pollinators was presented for each study site (Tab. 1). The plants were identified according to Jordanov (Jordanov, 1963-2012). In addition, the approximate abundance of the flowering plants in the neighborhood as

potential pollinator competitors was evaluated in accordance with their functional flower morphology with reference to the methods of Faegri and van der Pijl (1971, **Tab. 2**).

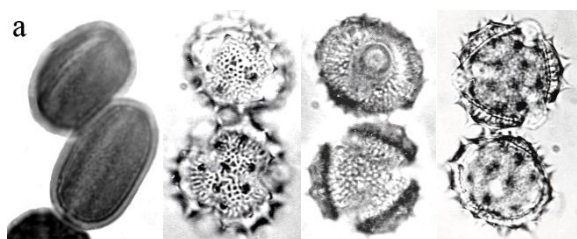


Fig. 2. Pollen of *Onobrychis pindicola* (a) and Achillea/ Aster type (others)

Analysis of the pollen loads

Bumblebees with full pollen loads (N=70) were individually collected from the flowers of *Onobrychis pindicola*. Additionally, bumblebees with full pollen loads were collected from the flowers of *Hypericum tetrapterum* (N=10) and *Oxytropis campestris* (N=9) in the near neighbourhood to check the flower constancy and food choice. Also, *Bombus pyrenaicus* workers (N=10) returning to their nest situated near Vihren hut (more than 1 km away from the patches of *Onobrychis pindicola*) were observed for one hour and those with full pollen loads were collected for pollen analysis. The collected bumblebees were immobilized in a tube in order to extract their pollen load and then immediately released. Pollen contamination was avoided by catching each bumblebee separately and pollen loads placed individually in jelly capsules. Each pollen load was transferred to eppendorf and mixed with glycerine and fuxin diluted in alcohol to loosen the clumps of pollen grains and homogenise the sample. Then the pollen sample was transferred to a microscope slide. Pollen identification (Faegri et al., 1989) and counting (at least 1000 pollen grains) were conducted under light microscope “Amplival” Carl Zeiss Jena (**Fig. 2**).

Data analysis

Descriptive statistics of central tendency were used to evaluate the calyx tube depth. One-way ANOVA was applied for data analysis to examine differences in the calyx depth within different subpopulations (study sites).

Bumblebee activity was measured by calculating the index of

visitation rate (IVR) as the quotient of the number of pollinators recorded and the minutes of observation multiplied by 60 minutes (Dafni, 1992). These data were used to assess which bumblebees pollinate *Onobrychis pindicola*, and to identify which plants compete for these pollinators with *O. pindicola*.

Pollen loads composition was evaluated in percentage.

Results and discussion

Access to the nectar

Nectar is secreted at the base of the stamen sheet. It is easily accessible to wide spectra of bees, including short tongued bumblebees and honey bees (**Fig. 3**). The restriction is the depth of the calyx tube. The mean depth is 2.2 ± 0.03 mm (n = 275, min = 1.4, max = 3.4). The presumption is that in some subpopulations/study sites (e.g. alpine versus subalpine habitats, the ones located in Central Pirin versus the ones located in Northern Pirin) flowers with deeper calyx tubes dominate, while in others there are mainly shallower calyx tubes, which were rejected. There was not a statistically significant difference between groups as determined by one-way ANOVA ($F(2,24) = 2.331, p < 0.05$).

Pollinators – species composition, activity and foraging behaviour

Onobrychis pindicola is pollinated most actively by *Bombus pyrenaicus* (**Tab. 1**). This bumblebee species demonstrated an average index of visitation rate IVR=15.7. It ranged between IVR=6.0 and IVR=64.2 during the years of observations and at various study sites. The main factor which influenced the activity of visitations was the abundance of the

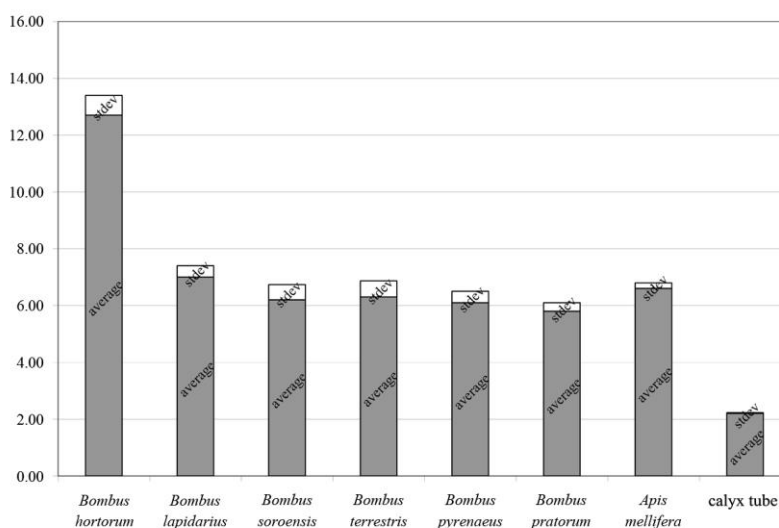


Fig. 3. Calyx tube depth (mean±stdev) of *Onobrychis pindicola* and tongue lengths of bumblebees (according to Goulson et al. 2008)

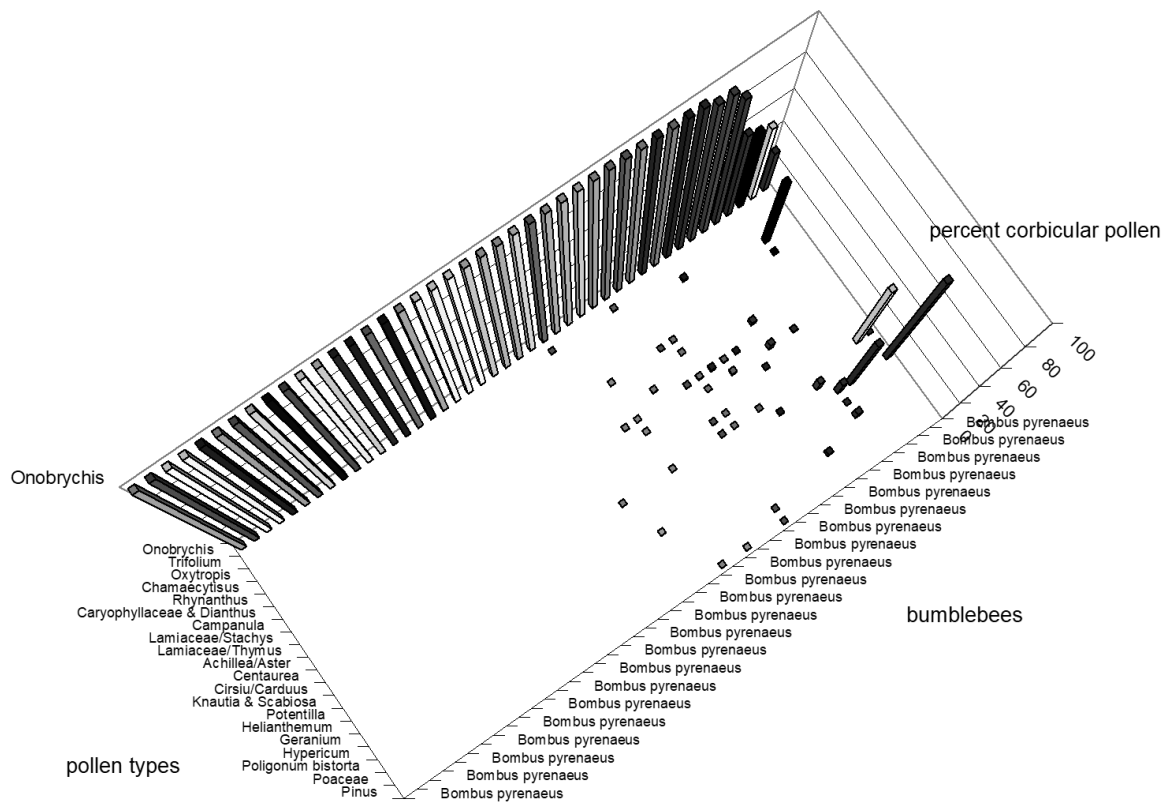


Fig. 4. Pollen loads of *Bombus pyrenaeus* pollinators of *Onobrychis pindicola* in the area of Vihren peak (study sites 12, 20, 10, 11, 3 and 4, see **Fig. 1** and **Tab. 1**).

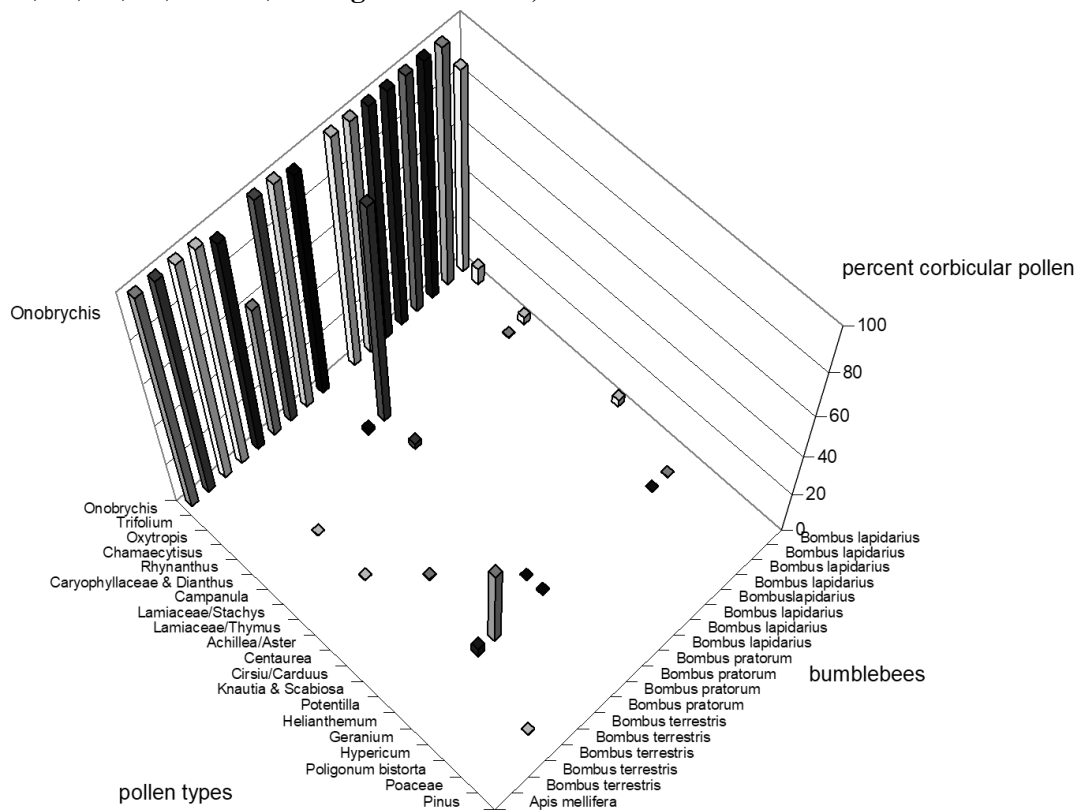


Fig. 5. Pollen loads of *Bombus terrestris*, *B. lapidarius*, *B. pratorum* and honey bee pollinators of *Onobrychis pindicola* in the area of Vihren peak (study sites 12, 20, 10, 11, 3 and 4, see **Fig. 1** and **Tab. 1**).

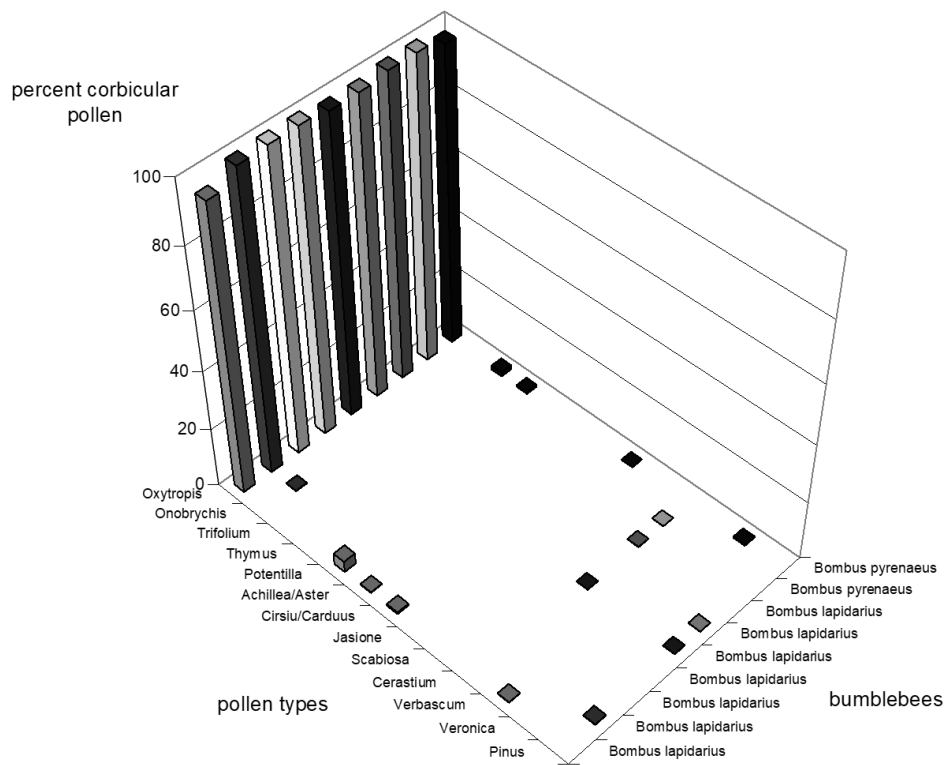


Fig. 6. Pollen loads of *B. lapidarius* and *Bombus pyrenaeus* pollinators of *Oxytropis campestris* in the area of Vihren peak (study sites 3 and 4, see Fig. 1).

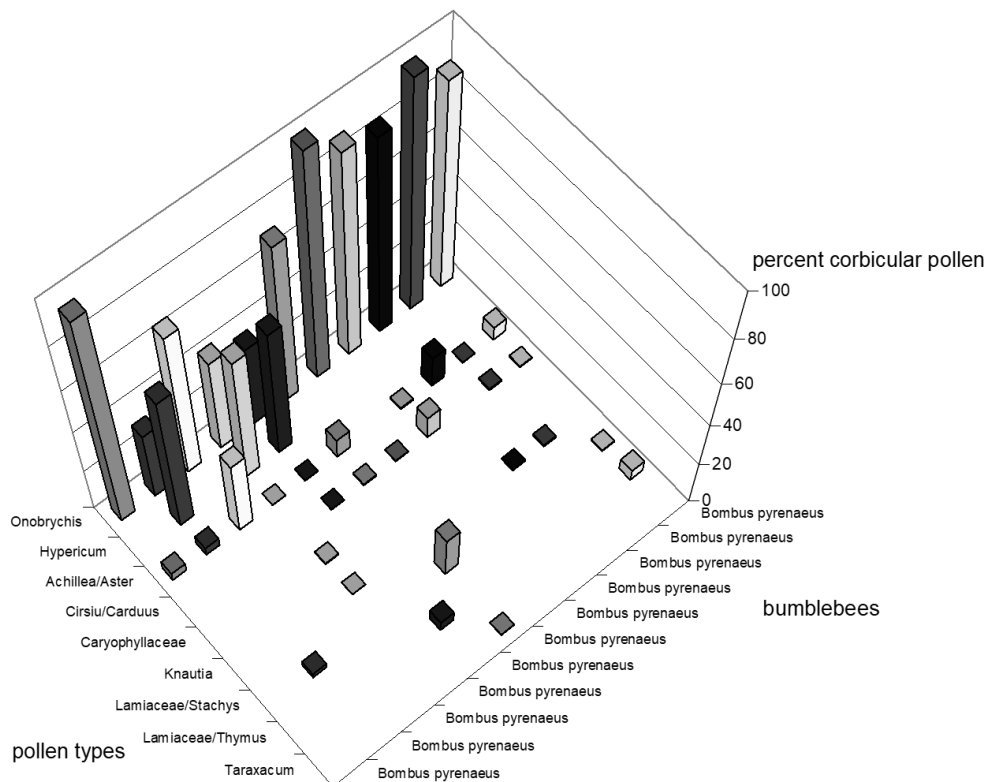


Fig. 7. Pollen loads of *Bombus pyrenaeus* pollinators of *Onobrychis pindicola* in the area of Kamenititza peak (study sites 15, see Fig. 1 and Tab. 1).

available flowers (**Tab. 1**). Visitation rates also differed between the years of observations and in cool, windy and cloudy weather the rate was lower. The highest peak of index of visitation rate, IVR=64.2 was recorded at study site 11 in the most favorable weather conditions after a rainy week, where the flowers available for foraging were evaluated as 20 000 000. Other bumblebees visited the flowers more or less actively (**Tab. 1**). These were *B. mastrucatus* group, *B. lucorum* group (as in the high mountain habitats *B. mastrucatus* specimens dominate while *B. lapidarius* are sporadic and *B. lucorum* specimens dominate while *B. terrestris* are sporadic) and *B. pratorum*. The second most active bumblebees in the flowers of *O. pindicola* were *B. mastrucatus/lapidarius* (average index of visitation rate IVR=5.7 and it ranged between 0.5 and 24.0, **Tab. 1**). During one single observation at study site 15 *B. mastrucatus/lapidarius* workers (IVR=24.0) were more active visitors of *O. pindicola* than *B. pyrenaicus* workers (IVR=14.4). *B. mastrucatus/lapidarius* basically favours other foraging plants and occasionally it switches to *O. pindicola* (e. g. *Oxytropis campestris*, *Carduus candicans*, *Hypericum tetrapterum*, **Tab. 1**). When other members of Fabaceae were present in the near vicinity such as *O. campestris* at study site 3. *B. mastrucatus/lapidarius* preferred their flowers. However, at study site 4, which is not far from study site 3., *O. campestris* were absent and *B. mastrucatus/lapidarius* was active in the flowers of *O. pindicola*. *B. pratorum* were moderately active in the flowers of *O. pindicola* (average index of visitation rate IVR=2.8 and it ranged between 0.6 and 5.4, **Tab. 1**). During their peak of activity in the flowers of *O. pindicola* (study site 15, IVR=5.4) they shared the same activity in the flowers of *Scutellaria alpina* and were twice as active in the flowers of *H. tetrapterum*. *B. lucorum/terrestris* were not active in the flowers of *O. pindicola* but they were not observed in the flowers of other plants as well (average index of visitation rate IVR=1.2 and it ranged between 0.2 and 3.0, **Tab. 1**). All bumblebees tended to visit more than one flower in the *Onobrychis pindicola* racemes. Field observations revealed high flower constancy. Once they visited *O. pindicola* they tended to follow its flowers. They switched to other plants on the same foraging trip, such as *S. alpina*, *Thymus* sp., *H. tetrapterum* rarely. They were observed to collect mainly nectar from the flowers of *Onobrychis pindicola* and transferred the pollen adhered on their bodies in their baskets in flight from ramet to ramet.

During our 60 minutes of observation on the nest of *Bombus pyrenaicus* we registered 59 workers – an individual landing each minute or landings each

two or three minutes sometimes two or three individuals simultaneously. Only 17% of these workers carried pollen loads.

Sporadic visits of honey bees were recorded at the lower altitude, subalpine study sites in the area of Vihren peak (sites 12, 20, 10 and 11, **Fig. 1, Tab. 1**).

Analysis of the pollen loads

High flower constancy, observed in the field, was confirmed by the pollen analysis. Once *Onobrychis pindicola* became their “major” source they kept to it (**Figs. 4, 5 and 7**). On average the pollen of *Onobrychis* in the pollen loads of bumble bee workers in the area of Vihren peak (study sites study sites 12, 20, 10, 11, 3 and 4, **Fig. 4 and 5**) was 94.7%. Of these pure, 100% *Onobrychis* pollen loads were 52%. Only in 5% of these pollen loads *Onobrychis* pollen was less than 50%. The most active visitors of *O. pindicola*, workers *B. pyrenaicus* had at the average 94.1% *Onobrychis* pollen in their loads (**Fig. 4**). Of these pure, 100% *Onobrychis* pollen loads were 50%. Sporadically in the pollen loads pollen of other plants (for instance *Chamaecitysus*-type pollen was as much as the *Onobrychis* pollen (**Fig. 4**). Only in one of the pollen loads was *Onobrychis* pollen less than 50% (**Fig. 4**). This particular pollen load was dominated by *Scabiosa* pollen which was not an abundant plant in the particular study. *Bombus mastrucatus/lapidarius* were not the most active visitors of the flowers of *Onobrychis pindicola* and they actually preferred *Oxytropis campestris*, but, where it was available, in the pollen loads of workers collected from the sainfoin flowers, on average 98.2% was *Onobrychis* pollen and 62% of the pollen loads consisted of pure *Onobrychis* pollen (**Fig. 5**). In comparison workers *Bombus mastrucatus/lapidarius*, which collected from *Oxytropis campestris* flowers, had on average 98.9% *Oxytropis* pollen and 0.2% *Onobrychis* pollen (**Fig. 6**). *B. terrestris/terrestris* workers also demonstrated high flower constancy with at average 74.4% *Onobrychis* pollen in their pollen loads (**Fig. 5**). Same was valid for *B. pyrenaicus* workers with at average 98.2% *Onobrychis* pollen in their pollen loads.

On average the pollen of *Onobrychis* in the pollen loads of bumble bee workers in the area of Kamenitza peak (study site 15, **Fig. 7**) was 73.3%. None of these pollen loads consisted of pure, 100% *Onobrychis* pollen. In this situation the content of *Hypericum* pollen (**Fig. 7**) was more often high. For comparison the pollen loads of *Bombus mastrucatus/lapidarius* and *B. pratorum* workers collected from *Hypericum tetrapterum* or *Stachys alpina* contained dominantly *Hypericum* pollen and sporadic pollen grains from *Stachys*, no matter in which species the bees foraged. This fact reveals that

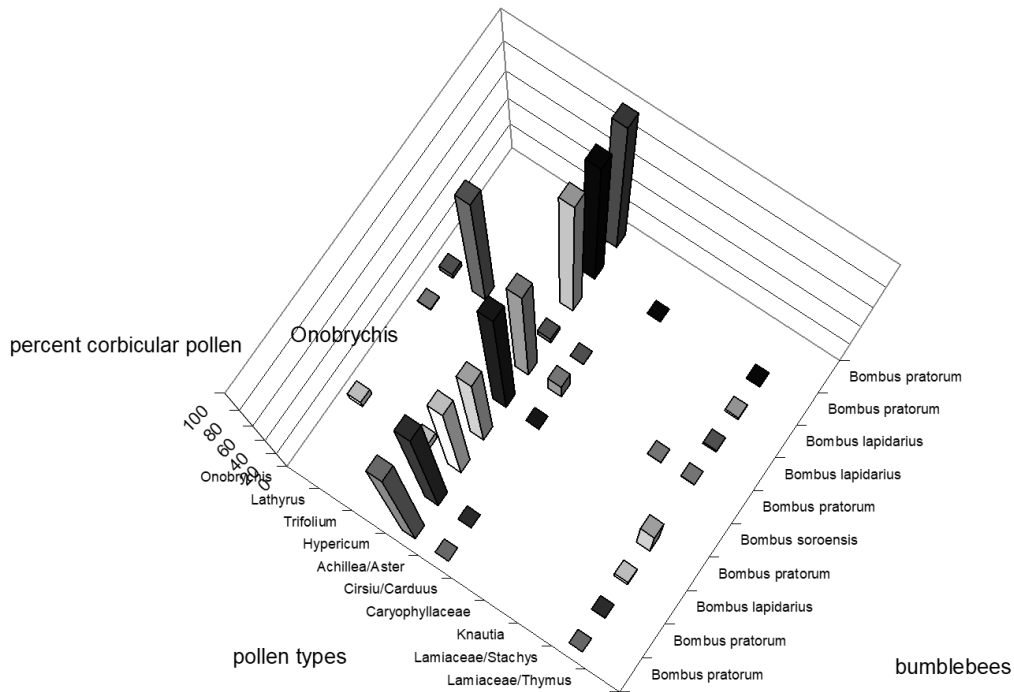


Fig. 8. Pollen loads of *Bombus* sp. div. pollinators of *Hypericum tetrapterum* in the area of Kamenititza peak (study sites 15, see Fig. 1 and Table 1).

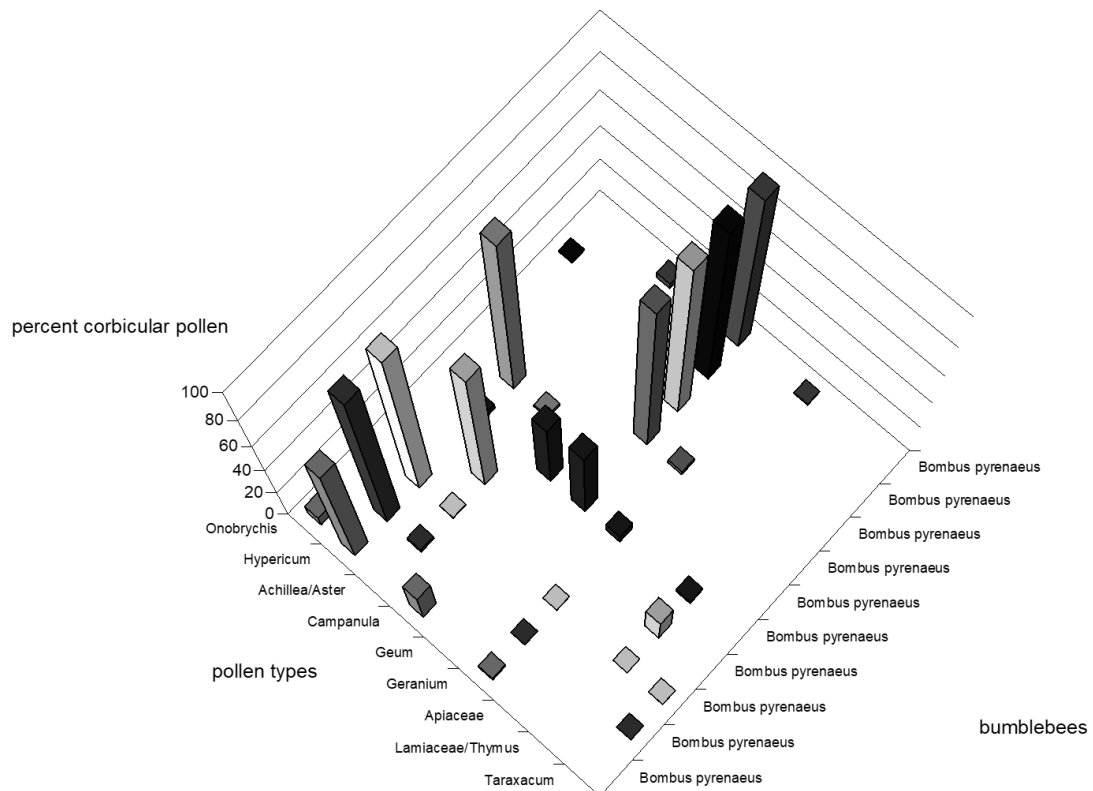


Fig. 9. Pollen loads of *Bombus pyrenaeus* workers returning to their nest situated near Vihren hut – more than 1 km away from the patches of *Onobrychis pindicola*.

the bees alternated between both species and used *Hypericum tetrapterum* as a pollen source and/or *Stachys alpina* as a nectar source. In some of the pollen loads of these bees *Onobrychis* pollen was present, even though in small amount (**Fig. 8**).

In a few cases the bumblebees' behaviour observed in the field was not well reflected in the pollen loads. For instance, two individuals, collected in the flowers of *Thymus* sp., had pollen loads dominated by *Onobrychis* pollen, while *Thymus*

pollen was absent. Obviously, the gullet blossoms like *Stachys alpina* and *Thymus* sp. provide nectar but not sufficient pollen resources to the pollinating bumblebees.

Workers of the same species were distributed among different foraging plant species at one and the same study site. They might be even members of one and the same colony, as was observed in the pollen loads of workers returning to the nest (**Fig. 9**). Individuals kept very high flower constancy for one foraging trip. This behaviour favours the reduction in competition for food resource.

Discussion

Onobrychis pindicola is pollinated most actively by *Bombus pyrenaicus*. Other bumblebees visited the flowers more or less actively. These were *B. mastrucatus/lapidarius*, *B. terrestris/terrestris* and *B. pratorum*. High flower constancy, which was observed in the field, was confirmed by the pollen analysis **Tab. 1, Figs. 4-9**). The attractant was primarily the nectar and the pollen was also collected from the body hairs.

Few plants compete for *Bombus pyrenaicus* pollinators with *Onobrychis pindicola*. At most of the study sites marked dietary differentiation was observed in the field and confirmed by pollen analysis (**Tab. 1 and 2, Figs. 4-9**). Only at site 15 niche overlap was observed and *O. pindicola* shared its *B. pyrenaicus* pollinators with *Knautia midzurensis*, *Carduus candicans* and *Thymus* sp. (**Tab. 1**). Interestingly these plant competitors do not belong to the same functional flower/blossom morphology class (**Tab. 2**).

Bumblebees are polylectic and generalist pollinators (Goulson, 2003; Goulson & Darvill, 2004; Goulson et al., 2005; Teper, 2005; Vanderplanck et al., 2014; Gogala, 2015; Sikora et al., 2016). They possess mental flexibility (ability of the brain to rapidly and effectively shift from one mental operation to another) and foraging bumble bees can task switch when they are faced with many different flower types and must decide when to switch between types (Beaulieu, 2013). Bumblebees tend to utilize plant resources in guilds and follow particular plants for foraging. Flower consistency has a lot of benefits for both plants and pollinators (Free, 1970; Heinrich, 1976; Thomson, 1981; Adams, 1985; Waser, 1986; Stout et al., 1998; Gegear & Laverty, 2005). Also, bumblebees appear to collect the majority of their pollen from a few plant species ('majors') and much smaller amounts from many others ('minors', as referred to by Heinrich, 1976). This tendency is well recognised in the literature, as is the tendency for the major pollen species to differ

between bumblebee species (e. g. Brian, 1951; Free, 1970; Heinrich, 1976; Carvell et al., 2015). Our results harmonize with these statements. Both our field observations and pollen analysis reveal that bumblebees tend to split and share plant resources, which reduces the competition in the mountain habitats of Pirin Mts. marble ridge. Competition reduction is achieved by bumblebees, by using many different strategies (Goulson & Sparrow, 2009). In the pollen loads where we found 1-7 pollen type other than *Onobrychis* the functional flower morphology was different from the flag type – gullet or dish/bowl (**Tab. 2, Figs. 3-7**). This is an adaptation to efficient pollination of more than one plant species by avoiding blocking the stigma with hetero specific pollen (Thomson, 1982; Galen & Gregory 1989; Jakobsson et al., 2009).

One niche dimension in which bumblebee species have long been known to vary is tongue length, which leads to differences in floral preferences between species. Those species that forage primarily on Fabaceae tended to have long tongues (Goulson et al. 2008). Clovers, however, are pollinated by short tongued bumblebees (Drobna & Ptáček, 2003). The nectar of the endemic *Onobrychis pindicola* is easily accessible to a wide spectra of bees, including short tongued bumblebees and honey bees. *Onobrychis pindicola* was actively visited by bumblebees and even honeybees and therefore it can be listed as a honey plant. Its low land relative *O. arenaria* is among top plants that provide good bee keeping value for honeybee bumbles and solitary bees (Jablonski & Koltowski 2004). *Onobrychis pindicola* receives the pollination service mainly from the short-tongued bumblebees *B. pyrenaicus*. Their high visitation rate and flower constancy toward this plant resource indicate a "task switch" toward this plant resource. Additional proof was the analysis of the corbicular pollen of workers coming back to their nest. *Onobrychis* pollen was detected even though present sporadically (**Fig. 9**). The nest was more than 1 km way from *Onobrychis pindicola* patches. Bumblebees are known to forage over wide areas (Osborne et al., 1999; 2008; Goulson & Stout, 2001). The fact that *Onobrychis* pollen was presented indicate that they favour and prefer this species. The workers brought to the nest various types of pollen. This does not mean a lack of information exchange between the colony members. On the contrary bumblebees are known to exchange foraging information (Plowright & Laverty, 1984; Dornhaus & Chittka, 2001; 2004; Mirwan & Kevan, 2013). The explanation lies with the different cells in the colony and brood care (Brian, 1951; 1954; Heinrich, 2004; Jandt & Dornhaus, 2009). The bumblebees' foraging over a wide area was confirmed also by the recorded

cases of pollen collection from plants which were not presented in close vicinity or are not cornucopias but form small patches of sporadic individuals (e.g. *Scabiosa columbaria*). The diet breadth of bumblebees is correlated with bumblebee abundance: rarer species tend to visit fewer flower species, after correcting for differences in sample size. The most abundant bumblebee species occupy distinct dietary niche space. Bumblebee species with tongues of similar length tend to have higher dietary niche overlap (Goulson et al., 2008) and vice versa (Teper, 2005). Among the group of abundant short-tongued species that commonly occur together there is marked dietary differentiation which may explain their coexistence (Goulson et al., 2008). Our field observations and pollen analysis confirm this statement. We detected dietary differentiation between the sympatric *Onobrychis pindicola* and *Oxytropis campestris*. The first pea flower was pollinated predominantly by *B. pyrenaicus* and the second one – predominantly by *B. mastrucatus/lapidarius* (**Tab. 1**, study sites 3 and 4, **Figs. 4** and **6**). During one single observation at study site 15, *B. mastrucatus/lapidarius* workers were more active visitors of *Onobrychis pindicola* than *B. pyrenaicus* workers. This indicates that the character of colonization is an important factor for the species composition of bumblebees pollinating *Onobrychis pindicola*, beside the mouth parts and flower morphology. Our record is in harmony with the findings of Bowers (1985) concerning the patterns of bumble bee (*Bombus*) species colonizing subalpine meadows.

Goulson and coauthors (Goulson et al., 2008) state that some bee species exhibited marked altitudinal preferences; *B. humilis* and *B. terrestris/terrestris* are predominantly found at the lowest sites, while *B. pyrenaicus*, *B. wurflenii* and *B. pratorum* are more abundant at high altitude. Our observations (**Tab. 1**) confirm that *B. pyrenaicus* and *B. pratorum* this in the Pirin Mts. At the same time we recorded *B. terrestris/terrestris* also at this high altitude and the highest activity was at our highest study sites. The presence of the honey bee is unusual at this high altitude. To our knowledge there are no apiaries in the near vicinity. Hives are beneath the coniferous forest belt. So, these workers were either members of a wild colony or they flew from a far distance. Our field observations were during the period of meadow mowing in the Pirin Mts. foothills.

Bumblebees show a tendency for rotation in the same direction around each inflorescence on successive visits, i.e., each individual tended to go either clockwise or anticlockwise (Kells & Goulson, 2001). We observed similar behavior in the inflorescences of *Onobrychis pindicola*, although

sometimes this was not clear, because they visited one or two flowers before move to another inflorescence/ramet.

It is clear from studies of population structure that most bumblebee species cannot be conserved by managing small protected ‘islands’ of habitat within a ‘sea’ of unsuitable, intensively farmed land. Large areas of suitable habitat are needed to support viable populations in the long term. These large areas need to be rich in Fabaceae (Goulson 2003, 2009). The mountain subalpine and alpine calcareous grassland of Pirin Mts. marble ridges offer good shelter for bumblebees. Bumblebees and their ecological specifics should not be neglected in management plans for the territories of National Park Pirin.

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