Autecological observations on *Oxytropis* species (*Fabaceae*) – two of them rare and endemic from Northern Pirin Mts, Bulgaria

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Abstract. The Pirin Mts. hosts more than 20 species endemic to the Pirin marble. Amongst these endemics are two alpine species of *Oxytropis*. The diploid *O. urumovii* is not locally frequent but the population occupies a large territory. The tetraploid *O. kožuharovi* is only known from a single locality from the northernmost part of the Pirin Mts, the Yavorov Anticline. A third alpine species, the hexaploid *O. campestris*, which has wide distribution in Europe also occurs in these mountains. We observed a number of microhabitat specifics of the three *Oxytropis* species in the Pirin Mts. Here we present details about the environment – slope, exposure, bed rock, soils, vegetation and phenology as well as notes on ex situ growth of *O. urumovii* and *O. kožuharovi*.

Key words: ex situ, microhabitat, *Oxytropis*, phosphorus, Pirin Mts, soil pH,

Introduction

Pirin horst is one of the most spectacular geological structures in Bulgaria. It is a neotectonic block structure that has inherited elements of Precambrian, Palaeozoic, late Mesozoic and Palaeogene structures. It is of Neogene-Quaternary origin. The rocks involved are extremely complex, dating from a wide variety of geological periods. They had mostly been metamorphically modified and folded in several deformation events. Much of the northern Pirin Mts is marble – Razlozhki and Sinanitsa anticlines (Zagorchev 1994, 1995a,b, 1998). Velčev & Kenderova (1994) separated three glaciations, Mindell, Riss and Wurm, on the basis of thermoluminiscence analyses of correlative deposits from the western foothills of Pirin.

There are more than 20 plant species endemic to the Pirin marble (Kožuharov 1976; Velčev 1984; Velčev & al. 1992). Amongst these Pirin endemics there are two alpine species of *Oxytropis*. The genus *Oxytropis* has about 300 species distributed through temperate, mountain and boreal regions of the northern hemisphere.

Some species in both the genera *Oxytropis* and the closely related and larger *Astragalus* (2500 species) are 'locoweeds', selenium accumulators, or contain the al-
kaloid swainsonine and thus are harmful to stock (De- 
meuov & al. 1998; Ralphs & James 1999; Ralphs & al. 
2000; Torell, & al. 2000; Gardner & al. 2001; Stegelmei-
er & al. 2001; Pfister & al. 2001). Lately it has been 
shown that there is a relationship between the endo-
phyte Embellisia spp. and the toxic alkaloid swainso-
nine in major Astragalus and Oxytropis locoweed spe-
cies (Ralphs & al. 2008).

There are three acaulescent alpine Oxytropis spe-
cies in Bulgaria (Kožuharov 1976). They are tap-root-
ed. Using morphological characters and molecular 
techniques, including RAPDs, ITS and trnL sequenc-
es, we compared O. urumovii Jáv. and O. kozhuharovi-
D.Pavlova, D.Dimitrov & M.Nikolova, two alpine Ox-
ytropis endemics to the North Bulgarian Pirin marble, 
with the neighbouring populations of the widespread 
O. campestris (Kožuharova & al. 2007). Two of them – 
O. urumovii and O. campestris (L.) DC. – have yellow 
flowers. The whole plant of O. urumovii, including 
the fruit, is rather densely covered with long whitish 
hairs and the green colour of the calyx is often suf-
fused with black. The indumentum of O. campestris 
usually is sparse and semi-appressed. In addition the 
flowers of O. urumovii turn reddish at the tip, it has a 
smaller fruit and in general it has a dwarfer and stiffer 
habit. The flowers of O. campestris turn blackish at the 
tip. It has a larger fruits and a rather lax and sprawl-
ing habit. O. kozhuharovii has purple flowers and 
most distinct feature is the long white hairs on the ca-
lyx which equal or exceed the calyx teeth (Kožuharov 
1976; Kožuharova & al. 2007).

O. urumovii is a local endemic for the calcareous 
part of the Pirin Mts and is a rare species in the Bul-
garian flora (Kožuharov 1976, Velčev 1984; Velčev & 
al. 1992). It is a diploid 2n=2x=16 (Krusheva 1986, 
Pavlova 1996). O. kozhuharovii is also a local endemic 
to the marble of the North Pirin Mts. It is a tetra-
ploid, 2n=4x=32 (Pavlova & al. 1999). O. urumovii 
and O. kozhuharovii are closely related, possibly pa-
rental to the third alpine species, O. campestris, which 
has a much wider range in Europe and North America 
and in Bulgaria grows on the marble rocks in Nor-
thern Pirin Mts and in a small area in the Rila Mts. It 
is a polyploid, 2n=6x=48, with ‘asymmetric karyotype 
compared to the other karyotypes’ (Pavlova 1996). 
Using morphological characters and molecular tech-
techniques, including RAPDs, ITS and trnL sequences, 
the endemics O. urumovii and O. kozhuharovii were 
compared with neighbouring populations of the wide-
spread O. campestris, O. halleri Bunge and O. dinari-
ca Murb. Oxytropis urumovii is a very distinct diploid 
species which might be ancestral to this group and 
could be regarded as a palaeoendemic. The tetraploid 
O. kozhuharovii is most closely related to O. prenja 
from the Dinaric Alps, Bosnia-Herzegovina, but is a 
larger plant with a different facies and indumentum. It 
is possible that it has evolved as an allotetraploid der-
ivative of O. urumovii and O. halleri. It is also pos-
sible that the circumpolar hexaploid O. campestris 
has evolved as an allohexaploid derivative of the dip-
loid O. urumovii and a tetraploid from the Balkans, 
such as O. kozhuharovii (Kožuharova & al. 2007). The 
O. urumovii IUSN category is “Vulnerable” and that of 
O. kozhuharovii is “Critically Endangered” (Dimitrov 
2009, Dimitrov & Kozhuharova 2009).

Simon (1958) conducted a study of the alpine vegeta-
tion in the Pirin Mts and published some associa-
tions and a new alliance. Also, stony vegetation of the 
alpine belt in the Pirin Mts was studied by Mucina & 
al. (1990). It has recently been reviewed by Bondev 
(1991) and Tzonev & al. (2009).

The aim of this study is to investigate the micro-
habitat characteristics of Oxytropis kozhuharovii, 
O. urumovii and O. campestris, namely slope, expo-
sure, bedrock, soils, and vegetation, as well as spatial 
distribution and phenology regarding their reproduc-
tive isolation.

Material and methods

Study sites

The field observations were conducted in the mar-
blesied karst region of the North Pirin Mts, namely 
in its impressive alpine area. The terrain includes the 
main watershed with Vihren peak and the next high-
est peaks around it, their slopes built of marble. The 
study sites are summarized in Table 1. Description of 
the soils and soil-forming processes of the study sites 
are result of long-term research by N. Ninov.

The period of investigation of wild populations 
was during the summers of 1995, 1996, 2001, 2002, 
and 2005. The ex situ observations were conducted 
during the period 2006–2010. The exact geographic 
location of all sites was determined using a global po-
sitioning receiver Garmin GPS 12, Datum WGS 1984, 
UTM projection. Elevation was double checked with 
an altimeter.
Habitat observations
Slope and exposure were recorded and described both in the field and using the global positioning system (GPS) methods.

The geomorphology of the Northern Pirin marble ridges is analysed from soil genesis point of view. Soil samples (two samples from each study site) were taken from the rooting zone of study plants. Each sample was taken from area of 20–30 cm² and 4 cm depth (Table 2). The soil characters were measured after a standard methodology at Newcastle University in January 2002. The volume of 10 cm³ of air-dry soil (scoop

Table 1. Sites from which Oxytropis were observed in the wild. Datum WGS 1984, UTM projection.

<table>
<thead>
<tr>
<th>Species</th>
<th>Waypoints (wp)/Sample number</th>
<th>Site</th>
<th>Altitude [m]</th>
<th>date</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. campestris</td>
<td>3</td>
<td>Vihren hut to Kabata</td>
<td>2410</td>
<td>8.7.01</td>
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<tr>
<td>O. campestris</td>
<td>5</td>
<td>Kabata</td>
<td>2640</td>
<td>8.7.01</td>
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<tr>
<td>O. campestris</td>
<td>7</td>
<td>Kazan, Vihren</td>
<td>2605</td>
<td>8.7.01</td>
</tr>
<tr>
<td>O. campestris</td>
<td>22</td>
<td>Vihren hut to Kabata</td>
<td>2200</td>
<td>1.8.01</td>
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<tr>
<td>O. campestris</td>
<td>19</td>
<td>Razlozhki Suhodol</td>
<td>2570</td>
<td>29.7.01</td>
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<tr>
<td>O. urumovii</td>
<td>8</td>
<td>Kazan, Vihren</td>
<td>2605</td>
<td>8.7.01</td>
</tr>
<tr>
<td>O. urumovii</td>
<td>19</td>
<td>Razlozhki Suhodol</td>
<td>2570</td>
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<tr>
<td>O. kozhuharovii</td>
<td>21</td>
<td>Kazan II, Vihren</td>
<td>2251</td>
<td>31.7.01</td>
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<tr>
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<td>16</td>
<td>Zhulti skali, Okadenski cirque</td>
<td>2170</td>
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<td>2210</td>
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<tr>
<td>O. kozhuharovii</td>
<td>38</td>
<td>Zhulti skali, Okadenski cirque</td>
<td>2320</td>
<td>9.9.05</td>
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</tbody>
</table>

Table 2. Soil samples – characteristics; Legend: (-) not presented in the sample, (+) sporadic or few, (+++) a lot of, (++++) mostly. Readings for pH were made at 20.5 °C.

<table>
<thead>
<tr>
<th>Oxytropis species</th>
<th>Locality</th>
<th>Sample number</th>
<th>Slope</th>
<th>Exposure</th>
<th>Weight volume [g/10cm³]</th>
<th>Roots &amp; straw</th>
<th>Sand</th>
<th>Pebbles</th>
<th>Water absorption</th>
<th>pH</th>
<th>mg P/kg soil</th>
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<tr>
<td>campestris</td>
<td>Beneath Kabata</td>
<td>22</td>
<td>10°</td>
<td>E</td>
<td>12.2</td>
<td>+</td>
<td>+++-</td>
<td>-</td>
<td>medium</td>
<td>6.73</td>
<td>20.05</td>
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<td>Beneath Kabata</td>
<td>22</td>
<td>10°</td>
<td>E</td>
<td>6.3</td>
<td>+</td>
<td>+++-</td>
<td>-</td>
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<td>5.91</td>
<td>20.52</td>
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<td>10–18°</td>
<td>SE</td>
<td>3.9</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>delayed</td>
<td>6.99</td>
<td>20.38</td>
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<td>Beneath Kabata</td>
<td>3</td>
<td>10–18°</td>
<td>SE</td>
<td>7.6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>slow</td>
<td>6.75</td>
<td>20.80</td>
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<td>5–10°</td>
<td>S</td>
<td>7.1</td>
<td>+</td>
<td>-</td>
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<td>5.82</td>
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<td>20°</td>
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<td>+</td>
<td>++</td>
<td>+</td>
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<td>7.79</td>
<td>21.62</td>
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<td>20°</td>
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<td>12.6</td>
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<td>++++</td>
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<td>Kazan I (higher)</td>
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<td>20°</td>
<td>NW</td>
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<td>8</td>
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<td>6.3</td>
<td>+</td>
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<td>7.55</td>
<td>20.95</td>
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the reduction with ascorbic acid of the phosphomolibdate formed when acid ammonium molybdate reacts with phosphate was measured spectrophotometrically at 880 nm. The amount of μg of phosphorus equivalent to the absorbances of the sample and the blank determinations were calculated from the standard graph. The difference was multiplied by 100 to obtain the quantity of extractable phosphorus in the soil [mg P/kg soil] (after Olsen & al. 1954; Murphy & Riley 1962). Soil pH characters were measured using a pH electrode and meter at about 20 °C (between 20.2 and 20.8 °C for each sample). Particular attention was paid to the comparison between the soils collected from the sites of the endemic *O. urumovii* and *O. kozuharovii* to those of the widespread *O. campestris*.

Associated vegetation was recorded in the close vicinity in order to check microhabitat specifics. The plants were identified after Jordanov (1963–1979), Velchev (1982–1989) and Kožuharov (1992, 1995). Approximate abundance evaluation of the plant species was done after Drude scale descendingly as follows: Soc. (sociales), Cop.3 (copiosae3), Cop.2 (copiosae2), Cop.1 (copiosae1), Sp. (sparsae), Sol. (solitariae) (Jaroshenko 1961).

**In situ and ex situ phenology observations**

Flowering periods of the three species were observed in the field. The ontogenetic ex situ development of seedlings was observed during the period 2006–2010. These seedlings were planted at the 2–6 true leaf stage in 2006, 2007 and 2008 in the experimental rock garden at the foot-hills of Pirin Mts near Dobrinishte village (Table 3, details are published in Kožuharova & Richards 2009). They were planted singly into the flower beds with fine marble gravel top-dressing. The experimental rock garden was established in 2006, in a hay meadow of approximately 500 m² near the river, situated at 865 m a.s.l., at N 41°48’80,9” and E 23°33’67,4” (WGS84) on the steepest part of the hay meadow with an exposure to the north-east. The place was chosen with consideration to several factors: i) close enough to the river for watering; ii) away from potential floods; iii) moderately shaded; iv) the snow lies relatively long here, protecting the plants from the spring frosts, and providing a cool microclimate in summer.

### Results

**Habitat specifics – soils and soil forming processes of the study sites, space distribution of the Oxytropis populations, micro-relief and vegetation**

The studied populations are localized in the criolithogenic belt. Here is found periglacial relief which is a result of crionivalic processes with periodical freezing and unfreezing of the soil and the weathering crust. The mean yearly temperature is –3 °C, the rainfall is about 1200 mm and 60–80 % of it is snow, which remains about 180 days. The mean snow cover is about 80 cm thick. The growth period lasts three to three and a half months, when the mean temperature is above 3 °C, but temperatures above 10 °C occur rarely. Due to the karst terrain there are no lakes (except a single very small one). The significant rainfall combined with low evaporation and steep slopes cause soils to have a high moisture status, although 90 % of the rainfall passes rapidly through the profile; 1 kg of soil absorbs about 3 kg of water, helped in part by a present

### Table 3. Seed germination and development of the seedlings. Legend: ♣ date of planting, * seeds collected in 2005 from wild populations, ** seeds collected in 2005 from wild populations.

<table>
<thead>
<tr>
<th>Years</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
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<td>♣</td>
<td></td>
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</tr>
<tr>
<td><em>O. kozuharovii</em> – Number of seedlings</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>2006*</td>
<td>25</td>
<td>14</td>
<td>11</td>
<td>10</td>
<td>10</td>
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<tr>
<td>2007*</td>
<td>70</td>
<td>47</td>
<td>18</td>
<td>12</td>
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<tr>
<td>2008*</td>
<td></td>
<td>120</td>
<td>80</td>
<td>40</td>
<td>33</td>
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<tr>
<td><em>O. urumovii</em> – Number of seedlings</td>
<td></td>
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<tr>
<td>2006*</td>
<td>14</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>4</td>
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<tr>
<td>2007**</td>
<td>38</td>
<td>20</td>
<td>6</td>
<td>4</td>
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</tr>
<tr>
<td>2008**</td>
<td></td>
<td>24</td>
<td>19</td>
<td>9</td>
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</tbody>
</table>
layer of dead vegetation. Regeneration processes are slow, so that the mean vegetation cover is about 50%.

Peak Vihren (2915 m) is the fourth highest peak on the Balkans (Fig. 1). It is narrow, bare, with steep rocky slopes, and in some places with vertical crags and cliffs. It is piled with marble boulders. The soil is primitive, poorly developed and its cover is rather loose. The southern slope of Vihren peak is very steep, bare and soilless. Under it is situated the wide grassy saddle named Kabata (wp 5, Table 1). The eastern part of Kabata is dissected by the cirque bearing the same name, formed in the glaciations of the Quaternary and covered with grass vegetation and scattered here and there dwarf pines. It goes down to the U-shaped valley of river Banderitsa, where one of the most massive glaciers in Pirin Mts flowed during the Quaternary. The southwestern slope of peak Vihren is formed by a smoothed cliff named Stenata which runs down more than 1000 meters into the Vlahina river valley. The northeastern slope is vertical cliff of about 1000 m, which runs down into the cirque named Kazan (meaning ‘cauldron’).

Towards the north-west is developed the main ridge of the marble massive. Here are the peaks Kute-lo 1 and Kuteło 2, Banski Suhodol, Bayuvi dupki, Razlozhki Suhodol, Kamenititza (Fig. 1). The ridge is dangerously narrow so that there is a part named Koncheto (meaning “The horse back” and it is no wider than that indeed).

The area of Zhaltite Skali (Yellow rocks) above Okadenski cirque is slightly isolated from the main ridge (Fig. 1).

The population structure of all *Oxytropis* species in the Pirin Mts is of a mosaic type. The populations of *O. urumovii* and *O. campestris* are partially sympatric. They grow together at some of the study sites. *O. kozhuharovii* is more or less isolated in space (Table 1, Figs 1a-d). The plants can be seen both on steep slopes and on a flat ground such as the “bottom” of Kazan II (Table 2). Even when the slope is steep the plants tend to grow on small flat surfaces that look like stairs. The plants are found on silty brown earth amongst marble blocks, sometimes in pockets of big rocks. Extractable phosphorus (P) in the soil is between 20.05 mg (site 22, *O. campestris*) and 23.89 mg (site 8, *O. urumovii*).

The studied populations of all *Oxytropis* species occupy habitats where the vegetation cover varies between 50% and 80%.

**Oxytropis urumovii.** The population grows strictly on the marble bed rock (Table 1, Figs 1a-d). The patches of the population are small and not dense. They are scattered within a comparatively larger territory. The plants can be seen both on steep slopes and on a flat ground. Extractable phosphorus (P) in the soil is 21.67 mg (min=20.22, max=23.89) (Table 2). *O. urumovii* is a member of a plant community dominated by *Festuca valida* (Uechtr.) Pénzes Soc. (sociales), *Sesleria korabensis* (Kumm. & Jáv.) Deyl Soc., and *Carex kitaibeliana* Degen Soc. Abundant also are *Daphne velenovskyi* Halda, (the area is notable for this restricted endemic, Halda 1981) *Cop.3, Anthyllis vulneraria* L. *Cop.3, Saxifraga ferdinandi-coburgi* KELLERER & Sünd. *Cop.3, Onobrychis pindicola* Hausskn. *Cop.3, Potentilla apernina* Ten. *Cop.3, Linum capitatum* Kit. ex Schult. *Cop.3, Acinos alpinus* Moench *Cop.3, Centaurea achtarovii* Urum. *Cop.2, Thymus perniciosus* (Velen.) Iljas *Cop.2, T. thracicus* Velen. *Cop.2, Helianthemum nummularium* Mill. *Cop.2, Rhodax canus* Fuss *Cop.2, Cerastium alpinum* L. *Cop.2, Aster alpinus* L. *Cop.1, Achillea agetatifolia* (Sibth. & Sm.) Benth. & Hook.f. *Cop.1, etc.*
Fig. 1. a, Topographic map of the marbleized karst region of North Pirin Mts with localities of the three *Oxytropis*; b, View of the man ridge the marbleized karst region of North Pirin Mts; c, Topographic map of the area of Vihren Peak with localities of populations of *O. urumovii* and *O. campestris*; d, Geology map of the marbleized karst region of North Pirin Mts; e, Topographic map of Okadenski cirque with the localities of populations of *O. kozhuharovii*; f, View of the habitat of *O. kozhuharovii*. Legend: d1, Central Pirin pluton (equigranular botite to hornblende-biotite granites); d2, Glacial deposit (boulders, pebble, gravel, sands); d3, Porphyroid biotite granites (Bezbog pluton) Assenovgrad Group, d4, Sitovo Group Lukoviza Gneis-shist, Shist Formation (biotite, gneisses, shists, marbles); d5, Dobrostan Marble Formation (massive and layered marbles); Oc – *O. campestris*; Ou – *O. urumovii*; Ok – *O. kozhuharovii*. 
**Oxytropis campestris.** The main part of the population of *O. campestris* forms a large dense patch at the southern side of Vihren peak in the area of Kabata where it is very close to the contact zone with the granites (Fig. 1d). The dense patches are usually seen on the big flat surfaces. Few plants are on steep slope but they also inhabit small flat surfaces that the terrain offers. Thus in the area of Vihren peak the population is more or less separated in space from that of *O. urumovii*. In the highest part of cirque Kazanite together with *O. urumovii* there are few plants. The largest part of its population grows on silty brown earth on the table land in the area of Kabata (Table 1; Figs 1c, d). Extractable phosphorus (P) in the soil is 20.69 mg (min=20.05, max=21.62; Table 2). The plant community of *O. campestris* is dominated by: *Festuca valida* Soc. and *Sesleria korabensis* Soc. Abundant are also *Onobrychis pindicola* Cop.3, *Cerastium alpinum* Cop.3, *Gentiana verna* L. Cop.3, *Thymus pernicicus* Cop.3, *T. thracicus* Cop.3, *Genista depressa* M.Bieb. Cop.3, *Acinos alpinus* Cop.3, *Rhodax canus* Cop.2, *Armeria alpina* Wild. Cop.2, *Antennaria dioica* (L.) Gaertn. Cop.2, *Alyssum cuneifolium* Ten. Cop.1, *As- ter alpinus* Cop.1, *Achillea ageratifolia* Cop.1, *Jasione laevis* Lam. Cop.1.

**Oxytropis kozhuharovii** is only known from a single locality from the northernmost part of the Pirin Mts (Figs 1a, c). The location is on marble, gneiss-es and gneiss-schist formations near glacier deposits (boulders, pebble gravels) and granites (Fig. 1d). The exposure of the patches is east and south east (Table 1). It forms one big patch (about three thousand individuals) and a couple of small ones consisting by 5–30 individuals. The total number of plants in the population is estimated to about 3170 individuals (as follows: wp 16=12, wp 37=3130, wp 38=30). Some of the plants grow on very steep slopes: practically part of the population is on a snow-slip gully. However as the other *Oxytropis* species they will occupy the flat “stairs” (Fig. 1f). *O. kozhuharovii* grows on a stabilized scree with fine silty brown earth amongst mixed marble and siliceous blocks. Extractable phosphorus (P) in the soil is 20.84 mg (min=20.72, max=20.95, Table 2). *O. kozhuharovii* grows in a plant community dominated by *Festuca valida* Soc., *Sesleria korabensis* Soc., and *Carex kitaibeliana* Soc. Abundant are also *Onobrychis pindicola* Cop.3, *Chamaecytisus absinthioides* (Janka) Kuzmanov Cop.3, *Daphne oleoides* Schreb. Cop.3, *Saxifraga ferdinandi-coburgi* Cop.3, *Anthyllis montana* L. Cop.2, *Anthemis vulneraria* L. Cop.2, *Potentilla apennina* Ten. Cop.2, *Jurinea mollis* (L.) Reichenb. Cop.1. *O. kozhuharovii* plants occupy the open soil patches between the big tufts of *Sesleria korabensis* and small shrubs of *Chamaecytisus absinthioides.*

**Phenology and lifespan observations**

In situ the three *Oxytropis* species have not only spatial but also slight phenological isolation. *O. urumovii* is in flower a bit earlier than *O. campestris* (Fig. 2).

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**Fig. 2.** Flowering periods of the three species in situ and ex situ.
Ex situ seedlings of *Oxytropis urumovii* and *O. kozhuharovii* were grown and seed germination and early stage development (rather vulnerable) were presented in another paper (Kozuharova & Richards 2009). Once planted in the experimental plot, usually at least half of the planted seedlings of *O. urumovii* and *O. kozhuharovii* lived through the first summer (Table 3). Overwintering was the next Rubicon for the seedlings (Table 3).

All plants of *O. urumovii* produced only leaves. In 2009 one individual planted in 2006 bloomed in June and produced mature seeds but then the whole plant died.

Two seedlings of *O. kozhuharovii* grown in 2006 bloomed in 2007 (the next summer after sowing the seed, with 7 and 8 flowering stems respectively). One of them died until the next spring but the other bloomed again in 2008 (with 5 flowering stems). Also, three plants grown in 2007 bloomed in 2008 (with 3, 4 and 6 flowering stems). In 2007 both plants had fruits. In 2008, the fruits were produced by only one of the five flowering plants. In addition, the flowering period of “plant 2006” was a week or two earlier than the “plants 2007”, although there were open flowers of at least two plants at a time.

**Discussion**

The high-elevation ecosystems are characterized by extreme temperatures and large diurnal variation in growing-season temperatures, in conjunction with high levels of ultraviolet radiation, accompany large variation in the amount of precipitation or in other words as harsh habitats (Bowman 2001, Bowman & al. 2002). Alpine tundra vegetation exhibits strong growth responses and changes in species composition when nitrogen or phosphorus (or both) is added to the vegetation (Theodore & Bowman 1997). Such experiments indicate that the vegetation is still constrained by nutrient limitation (Seastedt & al. 2004).

Rate of soil N and P supply varied greatly among collecting locations in the Niwot Ridge, Colorado at 3550 m a.s.l., approximately 10-fold for inorganic N 96.7 to 68.9 μg N bag⁻¹ d⁻¹), and 100-fold for P (0.017 to 2.37 μg P bag⁻¹ d⁻¹). In addition, it was recorded that foliar N concentration did not reflect variation in soil N supply and the possible explanation is the potential reliance of plants on organic N (small amino acids) to meet their nutritional demands (Bowman & al. 2003). In the case of *Oxytropis* species, plants well known with their symbiosis with nitrogen bacteria such as *Rhizobium* which are able to fix nitrogen (Smyth 1997, Novikova & al. 1994, Poinsot 2001 etc.) the soil N supply is probably not crucial.

On our study sites, the high elevation with harsh climate and marble terrain cause an extremely poor soil-forming process and scanty soil hidden between the marble boulders, rock cracks, grooves, and fissures. The nature of the marble weathering is the reason for fragmented soil cover, poor development and functioning, as well as poor interaction with the vegetation. The index of the potential bio-production is 0.1 – the same as in the tundra or in the desert. Thus most of the soils here have poor morphology and quality. They have a “cryo” temperature regime and are defined as Cryrendolls. Such soils are rare for Bulgaria. They occur only here in Pirin and restrictedly in Mt Slavyanka. Being formed on hard rock, they are defined in details at lower taxonomic level as Lithic Cryrendolls (ST) or Lithic Leptosols – Rendzic Leptosols (F.A.O.). Usually the rendzic leptosols contain a high quantity of pebbles. Their vegetation is grassy, represented by the genera *Festuca*, *Sesleria*, *Carex* etc. and rarely shrubby, so they are used as pastures. These soils often contain carbonate. They have high pH values and moderate supply of phosphorus, potassium, calcium, and magnesium. The content of humus is comparatively high. Despite the humid climate they are not acid due to the high content of carbonate in the marbles. In the soils developed on marbles are observed vertical belts – a specific caused by the geomorphologic conditions, which is autochthon.

The habitats belong to montane tall-herb, grassland, fell-field and snow-bed vegetation. These are alpine and sub alpine open calcicolous herbaceous and alpine calcicolous herbaceous communities near melting snow-patches. Here is found psychrophytous and cryptrophytous hecistothermal vegetation in the alpine woodless belt; calciphilous cryptrophytous grass formations *Kobresieta myosuroides*, *Cariceta kitaibeliana*, *Seslerieta korabensis* and small shrub formations *Dryeta octopetalae*, *Saliceta reticulatae*, etc. It dominates a limited number of phytocoenoses occurring at approx 2500 m alt. The category of these habitats is endangered to critically endangered. The habitats are included in Annex № 1 of BDA. The localities of this habitat are within the borders of Rila and Pirin Na-
tional Parks. Some of the most representative localities are in sites of the European Ecological Network NATURA 2000 in Bulgaria. (Bondev 1991; Assenov 2006; Tzonev & al. 2009; Roussakova 2011). On the slopes of peaks Vihren and Sinanitsa in the Pirin Mts, with inclination of 30–45° and altitude above 2500 m, the phytocoenoses are dominated by Sesleria korabensis. In some coenoses, the rare and relic species Carex rupestris occurs as a co-dominant, or with lower abundance. In some phytocoenoses the abundance of Carex kitaibeliana is high, while in others Sesleria coerulans Friv. is common. In some places these species are edificators (not only in the Pirin Mts). The local endemic to the Pirin Mts, Oxytropis urumovii, is a rare species (Roussakova 2011). It is true for O. kozhuharovii too and its population is even more restricted in space (Kožuharova & al. 2007).

We chose to use the scale of Drude as this approach has particular importance for the evaluation of those plant species that grow in close vicinity to the three Oxytropis species and bloom simultaneously with them. It is all with respect to the analyses of pollination ecology (competition for pollinators). It is well known that individual bumblebees have primary foraging specialities (their majors) and secondary foraging specialities (their minors). Minors are often bridged to new majors (Heinrich 1976). Thus Oxytropis urumovii shared pollinators with Anthyllis vulneraria and was “minor” for the same species of bumblebees but being less abandoned were also less attractive. The highest flower constancy of the pollinators of Oxytropis campestris is connected to their dense patches and suitable food resources. They are “majors” for their bumblebee pollinators. The bumblebees Bombus lapidarius were observed to follow strictly the inflorescences of O. campestris. Few of them visited Onobrychis pindicola on the same foraging trip. The two other plant species in close neighbourhood to O. campestris visited actively by bumblebees were Onobrychis pindicola and Jasione laevis. They both were visited by other species bumblebees and were not competitors for pollinators (Kožuharova 2000).

The patches of populations of each Oxytropis species are to some extent isolated in space (Fig. 1). This is especially true for O. kozhuharovii which basically have no space contact with the other two species, while in some places O. campestris and O. urumovii individuals grow sympatrically (Fig. 1). The floristic composition of plant communities of all Oxytropis species, is rather similar, although in each patch of their populations the individuals are surrounded by different plant species – differences refer to both qualitative and quantitative characteristics. It is worth a mention however, that O. campestris was not observed growing in close vicinity with Potentilla apennina. The differences of the habitats are more or less expressed in the pH, and basic rock. O. campestris and O. kozhuharovii are more associated with the contact zone with the granites while O. urumovii is strictly on the marbles. O. kozhuharovii occur at slightly lower altitudinal range compared to the other two species. Widest range with regard to altitude shows O. campestris.

Scarcification with sand paper was often applied for increasing the germination of legume seed (Astragalus, Hedysarum, Lupinus, Oxytropis) and the effect was significant: up to 100% (Kaye 1997). The seeds of four Astragalus species germinated at any temperature (13–34°C), if they were scarified, (Platikanov & al. 2006). Peak germination percentage for Astragalus australis var. olympicus occurred at 15–25°C alternating temperatures and at moisture availability with low water potential (distilled water) combined with scarification (Kaye 1999). Most mature test seeds of Oxytropis urumovii and O. kozhuharovii germinated within a couple of days if the seed coat was scarified (Kožuharova & Richards 2009). The hard seed testa replaces chemical primary dormancy of the seed, so that the seeds do not germinate too soon (in the autumn) but only when the weather warms during the following spring, when the testa will collapse after e.g. fungal degradation or possibly scarification on the steep slope and rough marble rock and pebble surface.

Some individuals of O. kozhuharovii in situ were of considerable size and thought to be many decades old (Kožuharova & al. 2007). Ex situ observations on the ontogenesis for four years revealed that the individual plant does not grow much on size. This observation corresponds to the hypothesis that vegetative propagation is not an option as plants do not branch as they send tap root. But seedlings can grow dense next to each other ex situ. Obviously in situ the examples of dense cover on 0.25 m² consists of different individuals.

The flowering period ex situ is about a month earlier (Fig. 2). The observed earlier blooming in situ of O. urumovii compared to O. kozhuharovii is obviously genetically determined as this flowering pattern is preserved ex situ even though a month earlier.
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References


