GUIDELINES for species conservation

Edited by Hana Pánková

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Institute of Botany, Czech Academy of Sciences, Průhonice

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About general guidelines for species conservation

The main aim of these guidelines is to review the scientific knowledge about the species and identify the gaps, and to summarize results from implemented management actions across the whole Europe. Based on these data, the best practices for the care about the species as well as their habitat are suggested. The guidelines thus describe activities focusing on habitat restoration as well as species-targeted activities such as ex-situ conservation or plant transplantation.

At the end of each book the simple Decision tree for practitioners is available. This decision tree describes very simply the most important species threats and suggests how to suppress them. It is, however, not a completely exhaustive description. Nevertheless, it serves as a useful tool for a quick evaluation of site or species population in the field.

The book contains the guidelines for following species:

Angelica palustris
Arnica montana
Dianthus gratianopolitatus,
Dracocephalum austriacum
Gentiana lutea
Gentianella bohemica

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GUIDELINES

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General guidelines for *Angelica palustris* conservation

Renata Ćušterevska, Cvetanka Stojchevska

Angelica palustris

General guidelines for *Angelica palustris* conservation

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Introduction

Angelica palustris is a rare and endangered plant species throughout its range. It is included in the Bern Convention and subsequently listed in Annex II of the EU Habitat Directive. In the European IUCN Red List of Threatened Species in 2011 is assessed as Data Deficient (DD).

In the Alpine biogeographical region, the conservation status is ‘Unfavorable-Inadequate’; the previous conservation status was ‘Unknown’. The future prospect is ‘Unfavorable-Inadequate’ and the trend is unknown. In the Continental biogeographical region, the status remains ‘Unfavorable-Inadequate’. In the Boreal biogeographical region, the conservation status is ‘Favorable’; the previous conservation status was ‘Unfavorable-Inadequate’. In the Pannonian biogeographical region, the current and previous conservation status is ‘Unfavorable-Inadequate’. The future prospect is ‘Unfavorable-Inadequate’ and the trend is stable (report under Article 17 of HD).

Habitat of *Angelica palustris* in North Macedonia (photo: Renata Ćušterevska)

SCIENTIFIC PART

1. General information about the species

1.1. Taxonomy

1.1.1. Nomenclature

The species *Angelica palustris* (Besser) Hoffm. (Gen. Pl. Umbell.: 174. 1814) from the Apiaceae family can be found as 2 synonyms *Ostericum palustre* (Besser) Besser (Enum. Pl. Volh., Defin. ed.: 94 (1822)) and *Imperatoria palustris* Besser (Prim. Fl. Galiciae Austriac. 1: 214. 1809).
It can also be found under several vernacular names: Marsh Angelica (English), Starodub łąkowy (Polish), Sumpf-Engelwurz (German), Барска Ангелика (Macedonian), Ostrík močiarný (Slovenian), Matočnik bolotnyj (Russian), Emaputik (Estonian).

1.1.1. Life form

Hemicryptophyte

1.1.2. Variability

The species might colonise meadow, herb, peat bog and reed bed communities with moderate soil moisture and nutrient content as well as medium light availability. Dittbrenner et al. (2005) have studied the abundance and fitness of individuals occurring in meadows characterized by various site conditions.

There is no information on variability throughout its range, but some geographic variability may occur because it extends over a large area. According to Slavik's findings (Slavík 1989), the plant is the most robust in the Moravian region. In cultivated conditions, the size of the plant is reduced and is about 80-100cm.

Several authors from different investigations from Poland (Czarna 1999, Bróż and Podgórska 2006, Towpasz et al. 2011, Krasicka-Korczyńska et al. 2014, etc.) pointed out that the water supply plays a major role in the maintenance of populations in the colonized sites. Krasicka Korczyńska (2008) investigated the course of phenological phases in relation to time of mowing and found that if the first cut occurs in mid-June, the individuals of *A. palustris* might bloom and produce seeds before the second cut, while if mowing is delayed and occurs in July, individuals lose the generative stems during mowing and the full blooming occurs in August. The investigations of Kostrakiewicz-Gierałt et al. 2018, concluded that regular mowing has a beneficial effect on the state of populations of and the formation of highly productive generative stems with substantial numbers of inflorescences and infructescences in abandoned meadows may promote successful seed dispersal and an escape from an unfavorable site.

1.1.3. Karyology

Angelica palustris is a diploid species with 2n=22 chromosomes and a nuclear genome size of 2C= 7143.06 pg (Šmarda et al. 2019). Genomic GC content is 38.5 % (Šmarda et al. 2019).

1.1.4. Genetics and genomics

The results from investigations from Germany (Dittbrenner et al., 2005) indicated that have strong population differentiation from a short-lived species with limited seed dispersal capacities that had never been covered in Eastern Germany. Consistently the geographic differentiation has been not reflected in the RAPD profile. Significant correlations have been noted between population size and the percentage of polymorphic loci ($P < 0.05$) and genetic diversity ($P < 0.05$). An analysis of seed production has shown positive relationships between average seed number and levels of genetic variation. These results support concerns regarding the loss of genetic diversity in endangered plant populations because this process might have harmful effects on reproductive fitness. (Dittbrenner et al., 2005).

1.2. Species distribution and conservation status

1.2.1. Species area

The plant is native and occurs in Eastern Europe and Western Asia. According to Euro+Med Plantbase, this species is distributed in the territories of Central, Eastern Europe (Austria with Liechtenstein, Belarus, Czech Republic, Estonia, Germany, Hungary, Latvia, Lithuania, Moldova, Russia, Slovakia, Ukraine) and the Balkan Peninsula (?Bulgaria, Romania, Croatia, Serbia, Kosovo, Montenegro and North Macedonia). The territory of North Macedonia is the edge of the species' global range.

1.2.2. Occurrence, conservation status and threat in particular countries

A species with a European-West Siberian range (Meusel et al. 1978). The area includes the continental areas of the temperate zone of Europe and western Siberia, to the west it extends to Saxony, Thuringia, Mecklenburg, Brandenburg and Bavaria. Its occurrence is also isolated in the Czech Republic and in western Slovakia. The furthest south is an isolated range in Serbia (Suva Planina), Montenegro (Kolašin) and North Macedonia (St. Naum Monastery). Occurrence in Austria and Bulgaria is undocumented. Furthermore, the marsh Angelica is found scattered in Poland, where the number of localities increases in the direction from west to east, and in the Baltics - it is missing in Lithuania, in Latvia there are several localities around the Gulf of Riga, and it is more numerous in Estonia. The focus of the species' range lies in Belarus, Ukraine and Russia. Outside the Carpathian Arc, it occurs in Ukraine, Moldova, northeastern Hungary (only 2 recent localities) and Romania, where it also extends into the inner part of the Carpathian Arc, occurring in the Mures river basin. To the east, it extends in an isolated area to the southern Yenisei. The southern border in Asia lies in Kazakhstan (Slavík 1989).

Fig. 1. Distribution map according literature data

Angelica palustris is listed on Annex II of the Habitats Directive and under Appendix I of the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention).

According to published information on the website for EUNIS (from EEA), the species is protected under the following Natura 2000 sites:

Table 1. Protected Natura 2000 sites where *Angelica palustris* grows.

Country	Site name (direct link)
Czech Republic	Hrdibořické rybníky
Estonia	Pakri Väinamere Karuse-Linnuse Kabli Kastna Kihnu Lao Rannaniidu Luitemaa Sõmeri Tõstamaa Väikese väina Vesitükimaa Anne Ropka-Ihaste
Germany	Kiesbergwiesen bei Bergholz (südlich Löcknitz) Beesenberg Uckerseewiesen und Trockenhänge Eulenberge Oberückersee Sernitz-Niederung und Trockenrasen Heimsche Heide Rhinslake bei Rohrbeck Wiesen und Quellbusch bei Radegast Engelwurzwiese bei Zwintschöna Engelwurzwiese östlich Bad Dürrenberg Haßlebener Ried - Alperstedter Ried
Hungary	Kőrises - Jónás-rész Nyírábrányi Káposztás-lapos Nyírábrányi Kis-mogyorós

Hungary	Csohos-tó Létavértesi Falu-rét Fülöpi láprétek Hanelek Bátorligeti-láp Újtanyai lápok Piricsei Júlia-liget Apagyi Albert-tó Apagyi Falu-rét Napkori legelő Teremi-erdő Daru-rét Nyírség-peremi égeresek Biri Nagy-rét Penészleki gyepek
Latvia	Piejura Randu plavas Vecdaugava Jaunciems Lielupes grivas plavas
Poland	Puszcza Kampinoska Solecka Dolina Wisły Jezioro Gopło Dybowska Dolina Wisły Nieszawska Dolina Wisły Doliny Brdy i Stążki w Borach Tucholskich Lisi Kąt Łąki Trzęślicowe w Foluszu Ostoja Barcińsko-Gąsawska Równina Szubińsko-Łabiszyńska Solniska Szubińskie Błota Kłócieńskie Mszar Płociczno Włocławska Dolina Wisły Ostoja Poleska Pastwiska nad Huczwą Torfowiska Chełmskie Torfowisko Sobowice Dolina Sieniochy Uroczyska Lasów Janowskich Poleska Dolina Bugu

Poland	Łąki nad Szyszłą Dolina Łętowni Przełom Wisły w Małopolsce Dolina Wolicy Nowosiółki Pawłów Kamień Adelina Dolina Górnej Siniochy Doliny Łabuńki i Topornicy Bystrzyca Jakubowicka Dolina Dolnej Tanwi Uroczyska Lasów Strzeleckich Ostoja Barlinecka Dolina Rawki Polany Puszczy Bolimowskiej Dębnicko-Tyniecki obszar łąkowy Ostoja Nadbużańska Pakosław Ostoja Nadliwiecka Starodub w Pełkiniach Dolina Biebrzy Ostoja Nidziańska Dolina Górnej Pilicy Ostoja Kozubowska Ostoja Stawiany Ostoja Szaniecko-Solecka Dolina Drwęcy Ostoja Lidzbarska Dolina Noteci Ostoja Nadwarciańska Ostoja Wielkopolska Rogalińska Dolina Warty
Romania	Bazinul Ciucului de Jos Călimani - Gurghiu Câmpia Careiului Insulele Stepice Şura Mică - Slimnic Sighișoara - Târnava Mare Tinovul Apa Roșie Turbăria de la Dersca Pădurea și Lacul Mărgineni
Slovakia	Zahorske Pomoravie

The percentage of species population covered by the network was estimated by comparing the population size within the network and the total population size in the biogeographical/marine region.

Table 2. Percentage of coverage by Natura 2000 sites in biogeographical/marine region

	ALP	BOR	CON	PAN
CZ			95	
DE			100	
EE		76		
HU				92
LV		100		
PL			74	
RO	100		100	100
SK				0

In the latest version of the European Red List of Vascular Plants (Directorate-General for Environment (European Commission) et al. 2011) *A. palustris* is listed in the DD (data deficient) category due to lack of information for the species.

This taxon is included in several National Red Lists:

Belarus: Vulnerable (Ermakova 2005)

Croatia: Data Deficient (Nikolić and Topić 2005)

Czech Republic: Critically Endangered (Grulich V., 2017)

Germany: It is classed as Endangered in Germany (Bundesamt für Naturschutz 2010).

Hungary: Strictly protected and Endangered (Király 2007) due to the small area in Hungary. All populations are in Natura 2000 areas.

Slovakia: The species occurs in one Natura 2000 site covering 100% of its localities and it is classified as Critically Endangered (Mereďa and Hodálová 2011).

Russia: Categorized Least Concern (I. Illarionova pers. comm. 2010).

North Macedonia: Critically Endangered (Matevski et all., 2019)

In Ukraine, it is protected in a hydrological reserve (Zokarnice Vorozbjanslij) and in a landscape reserve (Zokuznik Verchnjojesmonskij).

According to the Report under the Article 17 of the Habitats Directive for the period 2007–2012, in the Alpine biogeographical region, the conservation status is 'Unfavourable-Inadequate'; the previous conservation status was 'Unknown'. The future prospect is 'Unfavourable-Inadequate' and the trend is unknown. In the Continental biogeographical region, the status remains 'Unfavourable-Inadequate'. In the Boreal biogeographical region, the conservation status is 'Favourable'; the previous conservation status was 'Unfavourable-Inadequate'. In the Pannonian biogeographical region, the current and previous conservation status is 'Unfavourable-Inadequate'. The future prospect is 'Unfavourable-Inadequate' and the trend is stable.

1.3. Biology and ecology

1.3.1. Phenology

Flowering of the plant occurs after 2–3 years and takes place between July and August. The species is self-compatible (East 1940). Mericarps (hereafter referred to as seeds) do not show morphological adaptations to special dispersal agents, but are nonetheless able to float (Korneck et al. 1996). Seed germination occurs between April and May (Rybka & Vrbicky 2002).

1.3.2. Life form and strategy

The species is hemicryptophyte (biennial or perennial) with surviving buds on aboveground shoots at the level of the ground.

C-R strategist capable of resisting the competition of other meadow species in occupied habitats due to its growth and relatively fast biomass formation. The transition from C to R strategy is due to the monocarpic nature of the plant and the large production of diaspores, which is related to the need to regularly renew populations from diaspores. Monocarpy also causes numerous population fluctuations due to different climatic conditions in individual years. The germination time is probably relatively short, but the application of the species in the seed bank has not yet been clarified.

A. palustris (Besser) Hoffm., Gen. Umb. 174 (1814) (Ostericum palustre (Besser) Besser). Biennial to perennial up to 120 cm. Leaves 2- to 3-pinnate; sheathing bases well-developed; lobes coarsely serrate; base frequently oblique. Umbels terminal and lateral, with numerous rays. Bracts 0-3, caducous; bracteoles numerous, linear-lanceolate, with a whitish margin. Sepals well developed, broadly ovate, whitish. Petals white. Fruit 5-6 mm, ovate-elliptical, with prominent dorsal ridges (Flora Europaea ed. 2).

Fig. 2 Angelica palustris habit (photo: Renata Ćušterevska)

1.3.3. Reproduction

1.3.3.1. Generative

The flowers of *Angelica palustris are* small, white and arranged in compound umbels consisting of 8-30 umbellets. Involucral bracts are missing; involucels of bracelets are numerous. The central umbellet is larger than the laterals but seated in a shorter ray. Monocarp - flowering usually in the second year, less often in the third year and rarely in suboptimal conditions even later. If the plant is already in the reproductive ontogenetic phase, it creates a replacement inflorescence even after the stem has been bent several times. Bisexual flowers with coenocarpous gynoecium, allogamous - entomogamous (discus epigynus secreting nectar), pollinated mainly by hymenopterous insects including wasps, moths, some beetles and also butterflies. Autogamy is prevented by pronounced protandry, the stamens ripen in two periods (first the median and two from the outer circumference, then the remaining two). Maturation of anthers takes three weeks to a month (Slavík 1989). The fruits are schizocarps; the elliptic seeds are characterized by flattened backs and two broad wings (Koczwara 1960). The plant is self-compatible and reproduces by seed;

The longest known lifespan of diaspores is three years, however, germination significantly decreases and diaspores fall into secondary dormancy, which is only interrupted by longer-term stratification. Germination takes place best in the light. Achenes do not germinate in water. Optimal laboratory temperatures for germination are 30/10oC. Under natural conditions, part of the achenes (10-20%) germinate already in the fall, the remaining part only in the spring, while stratification is not necessary, but the development of the embryo is beneficial and germination is faster after stratification. Non-stratified achenes take considerably longer to germinate, but overall germination is not significantly reduced (Slavík 1989, own observation).

1.3.3.2. Vegetative

Vegetative reproduction is not known to occur (Kostrakiewicz-Gieralt et al. 2018).

1.3.4. Habitat

Angelica palustris might colonise meadow, herb, peat bog and reed bed communities with moderate soil moisture and nutrient content as well as medium light availability. It grows in neutral or slightly alkaline, nutrient-rich soils with a high content of organic material.

The species is mainly found in meadows such as fen meadows and bogs with *Betula pubescens*. In Ukraine, it grows in wet bogs and meadows, in ecotones among alder forests and bogs, in flood-plains of rivers as a component of the communities of *Molinietum coeruleae*, and *Caricetum acutiformis* associations (Vinichenko 2006). In Slovakia, the species occurs on wet, often fen, meadows on neutral to moderately alkaline, mineral-rich soils, with high organic carbon and calcium content and sometimes in moderately salted soils, in the lowland vegetation belt. The species requires a high underground water table but does not tolerate long-term flooding (Mereďa and Hodálová 2011). In North Macedonia, it develops in shaded areas, right next to the springs of the river, under the wide canopy of the *Alnus glutinosa* stems (Matevski, 2010).

According to the IUCN habitat classification, the plant belongs to habitats 4.4 – Grassland (Temperate) and 5.1 – Wetlands (inland) – Bogs, Marshes, Swamps, Fens, Peatlands.
According EUNIS classification, *Angelica palustris* belongs to:

E2.252 Moesio-Thracian hay meadows

E3.43 Subcontinental riverine meadows

E3.51 [Molinia caerulea] meadows and related communities

D4.1 Rich fens, including eutrophic tall-herb fens and calcareous flushes and soaks.

D5.2 Beds of large sedges normally without free-standing water

The species within its range belongs to several habitats according to HD (Reports on the main results of the surveillance under article 11 for Annex II, IV and V species (Annex B) from Poland, Czech Republic, Latvia, Slovakia, and Estonia):

1630 - Boreal Baltic coastal meadows

6410 - Molinia meadows on calcareous, peaty or clayey-silt-laden soils (Molinion caeruleae)

6440 - Alluvial meadows of river valleys of the Cnidion dubii

6450 - Northern boreal alluvial meadows

6510 - Lowland hay meadows (Alopecurus pratensis, Sanguisorba officinalis)

7210 - Calcareous fens with Cladium mariscus and species of the Caricion davallianae

7230 - Alkaline fens

Using the Ellenberg Indication Values (EIV) obtained from unweighted averages of phytocenological images for species of the FloraVeg database, the general ecological requirements of the *A. palustris* can be characterized as follows:

- light: EIV 7 – half-light plant, mostly occurring at full light, but also in the shade up to about 30% of diffuse radiation incident in an open area
- temperature: EIV 5.8 – moderate heat indicator, occurring from lowland to montane belt, mainly in submontane-temperate areas
- moisture: EIV 8.2 – transition between well moistened, but not wet soils and often soaked, poorly aerated soils
- pH reaction: EIV 6 – moderate to slightly acidic soil
- nutrients: EIV 7.5 – the species occur at nutrient-rich sites
- salinity: EIV 0 – the plant is salt intolerant, glycophyte

Angelica palustris is a heliophyte, that mainly grows in direct sunlight, less in shade up to 30% of full sunlight. Thanks to its height, it can reach the required amount of light in meadows without any problems, in bushes it grows only until the shrub layer is fully involved (Ellenberg 1991, Slavík 1989).

In terms of heat needs, the species is fairly eurythermal, and tolerates severe frosts (adaptation to continentality). In the Czech Republic exclusively in warm areas, but this is probably due to pedological requirements (Ellenberg 1991, Slavík 1989).

It is hygrophyte - a type of moist soil with a permanently high groundwater level. It is not adapted to longer-term flooding, nor stronger drying of the rhizosphere. It has been verified in cultivation that in conditions of longer-term flooding it stagnates in growth and even dies. In Poland observed in a wetland with *Carex appropinquata* SCHUMACHER (Ellenberg 1991, Slavík 1989).

Marsh Angelica grows on black soil and bog soils with a high content of organic carbon and calcium richly supplied with nutrients. Soils with neutral to slightly alkaline pH. It also grows on soils with moderate salinity. Since it does not tolerate long-term flooding, it probably also has higher requirements for soil air content (Ellenberg 1991, Slavík 1989).

1.3.5. Biotic interactions

1.3.5.1. Herbivory
There is no information, herbivory of common rodents, hares and ungulates can be assumed.

1.3.5.2. Pollination
Flowers of this taxon were visited by over 81 species of anthophilous insects derived from 5 taxonomic orders, indicating the presence of a generalised pollination system. However, detailed analyses of the frequency of insect visits, pollen loads and insect behaviour on inflorescences suggest that the plant is chiefly pollinated by large Dipterans, predominantly large Syrphid flies and Muscoid flies, that together are often responsible for over 90 % of total pollinations (Zych et al., 2014).

1.3.5.3. Symbiosis
It is not known and is not mentioned in the literature.

1.3.5.4. Antagonism
Plasmopara umbelliferarum (CASP.) SCHRÖTER ex WARTENW has been detected several times in the Czech Republic. Powdery mildew *Erysiphe umbelliferarum* DE BARY was also detected in cultivation in Průhonice (Slavík 1989). In Olomouc cultivation, this pathogen is quite common - it appears practically every year during the end of July and especially in August, and leads to earlier introduction of plants. Vital achenes are often not formed. For example, there was a very serious incidence in 1995 and again in 1996, while in 1997 the incidence was weak.

In 1997, in the third decade of July, the occurrence of an unspecified representative of the genus *Fusarium* was detected in Olomouc cultivation, which was manifested by the falling of entire flower-bearing stems approximately 0.5-0.7 m above the ground. After treatment with a fungicide, it disappeared. During the cultivation of the pathogen, the occurrence of a rather saprophytic fungus of the genus *Aphanomyces* DE BARY was also detected (Rybka, 2000).

1.4. Species threats

1.4.1. Current
Although *Angelica palustris* is a widespread species, due to habitat loss, its populations are generally small, fragmented and isolated, and with reduced biological vitality.

The main threats are modification of the hydrographic functioning of watercourses such as drainage and management of water levels, and canalization. The modification of cultivation practices, such as the abandonment of pastoral systems, an increased use of pesticides and fertilizers, leads to vegetation succession and increased competition. Habitat conversion into forest plantations or urban areas poses problems to this taxon (Commission of the European Communities 2009, Mereďa and Hodálová 2011).

Threats		Timing	Stresses	
1. Residential & commercial development	1.1. Housing & urban areas	Ongoing	1. Ecosystem stresses	1.1. Ecosystem conversion 1.2. Ecosystem degradation

Threats		Timing	Stresses	
2. Agriculture & aquaculture	2.2. Wood & pulp plantations 2.2.3. Scale Unknown/ Unrecorded	Ongoing	1. Ecosystem stresses	1.1. Ecosystem conversion 1.2. Ecosystem degradation
7. Natural system modifications	7.3. Other ecosystem modifications	Ongoing	1. Ecosystem stresses	1.1. Ecosystem conversion 1.2. Ecosystem degradation
			2. Species Stresses	2.3. Indirect species effects 2.3.2. Competition
9. Pollution	9.3. Agricultural & forestry effluents 9.3.1. Nutrient loads	Ongoing	1. Ecosystem stresses	1.2. Ecosystem degradation
			2. Species Stresses	2.3. Indirect species effects 2.3.2. Competition
	9.3. Agricultural & forestry effluents 9.3.3. Herbicides and pesticides	Ongoing	1. Ecosystem stresses	1.2. Ecosystem degradation
			2. Species Stresses	2.3. Indirect species effects 2.3.2. Competition

Table 3. Threats of Angelica palustris according the assessment for the European Red List from 2011

Direct disposal of bog biotopes

During the 20th century, bog biotopes were destroyed by conversion to agricultural land. The sites were drained and plowed. Currently, in some places, there is a return to meadow management.

Changes in the water regime of the habitat

The water regime of fen biotopes throughout the Czech Republic was disturbed the most during the eighties of the last century when there was widespread melioration of all waterlogged areas. For the Marsh Angelica, which requires moist soils with a permanently high groundwater level, drying out the bogs means certain disposal.

Chemicalization of agriculture

In this regard, Marsh Angelica is mainly threatened by the management of agricultural areas in the vicinity of the occurrence sites. The nutrient regime at the site can be affected by too high doses of fertilizers in the surrounding fields. The use of pesticides and other chemicals also has a negative effect.

Successive habitat changes

At present, the overgrowth of the habitat due to insufficient management is probably the biggest problem. In the absence of mowing, the Marsh Angelica would certainly give way to the competitive pressure of woody species.

The population of *Angelica palustris* at the St. Naum in North Macedonia develops in a very limited space and consists of a relatively small number of individuals. In addition, it is located in a zone with intensive tourist activities, especially in summer and autumn. Bearing this in mind, as well as other impacts on the wider area, it can be assumed that the species is seriously endangered.

The greatest threat to this species in the newly discovered sites, as well as in other sites in Poland, are changes in the use of wet meadows. An important factor that threatens the further existence of this species is also the drying of the area. Due to the significant drainage of meadows, especially in the vicinity of the villages of Chałupki and Rozbórz, the old-growth meadow forest was found only in the drainage ditches there and on their banks, where it still found moisture conditions suitable for its development (a similar situation was observed in the Iłżeckie Foothills near the village of Pomorzany - see NOBIS & PIWOWARCZYK 2004).

The proximity of roads, human settlements, and numerous post-agricultural wastelands dominated by ruderal plants resulted in an increase in the ruderal plants.

According to the Report under the Article 17 of the Habitats Directive for the period 2007-2012 the main pressures (activities which are currently having an impact on the species) classed as 'highly important' are shown in the Table 4.

Code	Activity
A03	Mowing or cutting grasslands
A02	Modification of cultivation practices
A04	Grazing by livestock
J02	Changes in water bodies conditions
I01	Invasive alien species
K02	Vegetation succession/Biocenotic evolution
I02	Problematic native species
J03	Other changes to ecosystems
K04	Interspecific floral relations

Table 4. Ten most frequently reported 'highly important' pressures

1.4.2. Future

Climate change is a major threat to the survival of the species. Since the species depends on the amount of groundwater, decreasing rainfall and increasing temperatures are a serious threat in the future.

The species covers different habitats. Some of them are semi-natural (meadows) and depend on human activity. The trend of abandoning the traditional methods of meadow maintenance (mainly regular mowing) poses a threat to the plant. With irregular maintenance, successive processes occur, during which plants from other vegetation classes dominate and the presence of meadow elements is significantly reduced. The abandonment of previously mown or grazed semi-natural grasslands can be expected to result in encroachment of woody species, decreased species richness, especially of management-dependent or low-grown plant species, and altered vegetation composition, as well as drastic changes in regional or national landscapes (Dupré and Diekmann, 2001, Pykälä et al., Prangel et al., 2023).

The emergence of invasive species and native problematic species is increasingly intensifying, primarily as a result of various human influences. If appropriate measures are not taken, the impact of these threats is expected to increase in the future.

The abovementioned Report shows that 'highly important' threats (activities expected to have an impact in the near future) are:

Code	Activity
A02	Modification of cultivation practices
A03	Mowing or cutting grasslands
J02	Changes in water bodies conditions
I01	Invasive alien species
I02	Problematic native species
K02	Vegetation succession/Biocenotic evolution
A04	Grazing by livestock
K01	Abiotic natural processes
K04	Interspecific floral relations

Table 5. Ten most frequently reported 'highly important' threats

1.5. Previous implemented management interventions

The information from the Table 6 is derived from the Member State national reports submitted to the European Commission under Article 17 of the Habitats Directive in 2013 and covering the period 2007-2012.

Code	Measure
2.1	Maintaining grasslands and other open habitats
6.1	Establish protected areas/sites
6.3	Legal protection of habitats and species
7.4	Specific single species or species group management measures
4.0	Other wetland-related measures
4.2	Restoring/improving the hydrological regime
6.0	Other spatial measures
6.4	Manage landscape features
7.0	Other species management measures
8.0	Other measures

Table 6. Ten most frequently reported 'highly important' conservation measures

Several methods can be used for maintain grasslands:

- Mowing ones/twice a year with mower
- Mechanical removal of woody plants: Woody plants can be cut by axe or pruning shears
- Annual spring burning with mechanical removal of woody plants
- Grazing: According to the instruction, “normal” grazing intensity should be applied using cattle, or a combination of cattle and sheep.

The most popular of mechanisms for establishing protected areas are:

- Government action, which can occur at a national, regional, or local level.
- Community-based initiatives by local people and traditional groups.
- Land purchases and holdings by private individuals and organizations.
- Protected areas established through co-management agreements.
- Development of biological field stations or marine laboratories.

Legal protection can be given to species on a national or international scale. Levels of protection vary for species and may include protection against killing, capturing, disturbing or trading, or damaging or destroying their breeding sites or resting places. Depending on the level of protection, activities such as development that are likely to affect protected species in these ways may be against the law and require licenses from a government licensing authority.

The development and application of a Species Management Plan that aims to ensure a stable population of the species for as long a period as possible. The plan envisages several main tasks that could be realistically implemented.

- Determining the current condition of the species at National level
- Identifying threats
- Improving the awareness of interested parties
- Taking measures to strengthen in-situ protection
- Taking measures for ex-situ protection
- Taking measures for continuous monitoring of vision

The realization of the planned tasks within the SMP will enable better protection of this endangered rare plant, based on the improved functioning of the responsible institutions, specific measures for ex-situ and in-situ protection, regular monitoring, as well as the higher awareness of the local population.

Restoring appropriate hydrological regimes is often an important component of fen restoration. The definition of what constitutes a favourable hydrological regime for a particular wetland feature is an important first but sometimes difficult step in this process. For example, hydrological conditions characterized for wetland plant communities of conservation value at a particular site may not necessarily be optimal for that community. Furthermore, there may be significant variation between sites or even locations within a site in terms of the hydrological conditions associated with a particular plant community, habitat or feature of conservation interest.

Czech Republic:
The Marsh Angelica occurred in the past in seven sites in the Polabí (Elbe River Basin) and Pomoraví (Marsh River Basin), presently only one site exists in Central Moravia – Hrdibořické rybníky. The species has been systematically replanted in this area since 1998 using an authentic plant material, which had survived in ex-situ rescue culture since the end of the late 1980s. Attempts to restore a second population in a fen near Černovír have not been successful yet.

The objective of the Rescue Programme Marsh Angelica is a long-term restoration of the species populations in at least two sites in the Czech Republic.

The Action Plan for Marsh Angelica was prepared in 1998 and approved by the Ministry of the Environment in 2000. Presently, an evaluation of its implementation and a preparation of next phases are in progress.

An important presumption for the success of this Action Plan is an appropriate management of habitats with occurrence of the Marsh Angelica. In these habitats, meadow biotopes are being mowed regularly. The mowing is carried out twice a year, in order to lower the competition of other meadow species (the first mowing being carried out in early June, the second one when the achenes have ripened).

In addition, many special measures are being implemented in order to strengthen the species. These measures are usually carried out by specialized companies or non-governmental organizations and consist of sowing seeds, planting saplings, mowing groups of plants and other actions. In the framework of these activities, also a research in biology of the species is permanently under way.

North Macedonia:
The location where this species develops in North Macedonia is located within the borders of Galicica NP and on the shores of Ohrid Lake, which is part of the UNESCO list as a world cultural and natural heritage.

Angelica palustris should have a special protection status, considering that the locality where it grows is part of the most significant and attractive tourist center in our country, especially in summer and autumn, when the plant has an optimal vegetative and reproductive period of development.

It is assessed as critically endangered at the National level according to the IUCN methodology.

The species is covered by In situ protection measures, as it is within the boundaries of NP Galichica. The commitment of the employees of the park is at an enviable level, not only in relation to this species, but the location where the marsh angelica is developing obviously requires taking additional protection measures, which would improve the results of this conservation method.

1.5.1. In-situ

Czech Republic:
The site near Hrdibořice has been protected as the Hrdibořické rybníky NPP since 1990. This is a bog area in the floodplain of the Blaty River with partially excavated areas flooded with water, which form the so-called Raška and Husák ponds. In the vicinity of these reservoirs, there are moist, once again grassy meadows on marshland, which have been mowed regularly twice a year since 1998.

Since 1998, the NPP Hrdibořické rybníky has been seeding and planting mudweed plants in pre-prepared cleared areas every year. There are several areas in the NPP where the population of *Angelica palustris* is spontaneously maintained, and only large-scale sowing of seeds is carried out on the site to spread it to other parts of the fen meadows.

The long-term problem of the location is that it is unstable the level of underground water. To ensure the stability of the hydrological situation, it would be advisable to verify the functionality of the melioration systems in the wider vicinity of the site and at the same time to determine the degree of influence of the groundwater level on the site by the system, especially the channel in the eastern part of the NPP.

Monitoring of the population of the species and underground water level.

North Macedonia:
The species is within the Galicica National Park. In 2023 Species Management Plan for *Angelica palustris* was developed. National Park Galicica has included the species within the regular Monitoring plan since 2023. Future researches on the biology and ecology of the species in North Macedonia are needed.

Poland:
Marsh Angelica has been protected by law since 2001 (Krasicka-Korczyńska 2008).

In 2004, in Poland agro-environmental programmes were introduced to protect the biodiversity in agricultural areas (Dz. U. No 174). The programme packages for different meadow types differ mainly in cutting dates to enhance the preservation of the population of birds and meadow plant species. In the PO1b package, the first cutting can be made starting from July 1. Cutting of meadows with *A. palustris* can considerably affect the possibility of annual fruit-bearing of this species, which is important to maintain the population size.

1.5.2. Ex-situ

Species survival requires appropriate conservation strategies that could not be taken without reliable information about species ecology and biology. Reproduction biology and habitat requirements are most important in this respect. Pollination strategies, germination behavior and seed dispersal seems to play a crucial role in population fitness and long-term survival of species. Moreover, seed morpho-anatomical characteristics, germination preferences and dormancy patterns are recognized as valuable indicators of species adaptation to the environment and ultimately for understanding evolutionary patterns (Baskin and Baskin 1998).

Ex-situ measures of *Angelica palustris* in Czech Republic:

Cultivation

Cultivation was founded in the Office of the ČSAV Průhonice (now the Office of the Academy of Sciences of the Czech Republic, v.v.i. Průhonice) Dr. By Slavík in the 1980s, the 20th century from achenes taken in the Hrdibořické rybníky locality. In 1992, part of the cultivated population was transferred to the Department of Botany of the Faculty of Natural Sciences of the Palacký University in Olomouc.

Every year during the winter, the seeds are sown on the surface of moist perlite and placed in a refrigerator for stratification (according to Krátký 1999), from where they are transferred to the cultivation greenhouse at the beginning of the growing season (end of February – Marsh). Seedlings are then transplanted into planters, and these plants are then used for planting on sites or for supplementing flower beds. In 2010, the entire cultivation population in Olomouc was transferred to the lands of the association Sagittaria in Olomouc-Křelov.

Cultivation of *Angelica* continues to take place separately in the experimental garden of the Botanical Institute of the Academy of Sciences of the Czech Republic in Průhonice.

In 2011, the population of the Marsh Angelica was established in the Botanical Garden of the Capital City of Prague, the reason being to educate the general public and popularize the issue of rescue programs.

Seed bank and testing the seed germination

Since 1993, the collection of fruits in cultivation in Olomouc has been taking place every year. The seeds were collected throughout the ripening pods, after collection they were dried at room temperature and then threshed and cleaned. After they were dried and cleaned, the seeds were stored in a refrigerator at a temperature of 4° C. Some of the seeds were used for sowing on localities and pre-growing plants, some of the seeds from selected years were stored in the Seed Bank of Endangered Species of the National History Museum in Olomouc (VMO) and some are stored in the working seed bank of the Sagittaria association. About 1 liter of fruits collected in the years 1994, 1996–2001, 2003–2012 are now stored in the working seed bank. There are seed samples with a volume of approx. 0.3 liters from the years 2010–2012 in the Museum of Natural History in Olomouc.

In Poland seeds of *Angelica palustris* from different sites were collected within the EU Funded Project „Ex situ conservation of endangered and protected wild plants in the western part of Poland".

In Germany, the species is a part of the Special Conservation Collections within the Botanical Garden of the Martin Luther University in Halle.

In the Republic of North Macedonia seed from the species was collected in the seed bank established last year within the Botanical garden at the Faculty of Natural Sciences and Mathematic in Skopje.

In vitro culture

According Klavina et al. 2004, tissue culture has been successfully used both for conservation needs and the propagation of plant material of rare and endangered plants species. Among the plants that were the subject of this research is the Marsh Angelica

2. Aims of rescue plans

The aim of this plan is to gather as much information as possible on *Angelica palustris* in order to improve its conservation status.

3. Suggestion of interventions

3.1. Site management

3.1.1. Mowing

In the investigations from Kostrakiewicz–Gieralt et al., 2018, the effect of meadow management type on selected population traits of the rare plant *Angelica palustris* in Poland was examined. The study has been carried out in a regularly mown meadow (Plot I), an abandoned meadow with medium high plants (Plot II) and an abandoned meadow with high plants (Plot III) situated in southern Poland (Proszowice Plateau). Observations conducted in the years 2010 and 2011 showed greater abundance of *A. palustris* in Plot I than in Plots II and III. This pointed to the significance of disturbance caused by mowing, which creates gaps in the plant cover and litter layer that become safe sites for A. *palustris* seedling recruitment and their subsequent growth. This research confirms the need for regular management of the plant's habitat by mowing.

Mowing may take place once or twice a year depending on traditional practices. Generally, the first mowing takes place in May–June, and the second in August–September. According the investigation of Krasicka–Korczyńska 2008, for the population of Angelica in the meadows used for hay to survive in a good shape, it seems best to maintain a traditional date of the first cut. However, it is recommended to delay the harvest of the second cut of the meadow sward until the end of August to allow fruit ripening and shedding.

Mowing, depending on the area, can be done with a tractor with a mower or by using a hand mower. The selected mowing areas should be properly marked so that the effectiveness of this measure can be monitored. The cut biomass should be removed from the site following applicable legislation.

3.1.2. Restoration of water regime

The fluctuating water level causes the death of Angelica's seedlings in dry years and their flooding and death in the lower parts of the terrain in wet years. Therefore, it is necessary to manage the water regime with appropriate techniques depending on the site on which the plant develops (drainage channels, intake channels, etc.).

3.1.3. Creation of new sites

In order to increase and restore the population of the plant, it is necessary to do detailed research to choose suitable places for their reintroduction. In this way, part of the lost localities can be "returned". Before implementing this activity, it is necessary to make a detailed action plan based on the results obtained from field research.

3.2. Species targeted intervention

3.2.1. Ex-situ conservation

The cultivation aims to maintain the gene pool of the *Angelica palustris* in ex-situ conditions and to produce seeds for growing seedlings, sowing, planting and preserving the species in the seed bank. For this purpose, appropriate facilities for the production of seeds and young individuals for sowing and potential planting in selected localities should be provided. This method is very important for the preservation of the fennel gene pool.

In the Czech Republic, there is a greenhouse for pre-growing seedlings, cultivation shelves with young Marsh Angelica plants, three cultivation tanks (120x80 cm), and a cultivation bed (area 10x25 m) with approx. 500-1,000 bog individuals. The annual production assumption of the cultivation population is approx. 2,000 young individuals and 3 liters of seeds.

The original cultivation of the Marsh Angelica in the experimental garden of the Bureau of the Academy of Sciences of the Czech Republic is important for the preservation of its gene pool. Cultivation is maintained by self-sowing, gardeners remove weeds and provide irrigation. A few plants are grown in pots and a few plants grow in the joints of the tiles between the beds, where they self-seeded.

As part of the cultivation monitoring, the number of flowering individuals will be recorded and the number of sterile individuals determined by a qualified estimate.

The collection of seeds for the National and International Seed Banks, large-scale sowing, and planting from the cultivated and native population.

Germination tests of seeds stored in the local seed collection are being carried out separately at the Museum of Natural History in Olomouc, tests are carried out after approx. 5 years of seed storage. The tests also verified the dependence of the successful emergence and growth of seedlings on soil moisture. The results confirm that high soil moisture is a critical moment for the successful emergence of seedlings. There is also a high correlation between soil moisture and further plant survival and flowering. Soil moisture around 60-80% appears to be a certain minimum limit for successful plant growth and development. In 2000, soil moisture was checked at both locations (Hrdibořice, Černovír) and fears were confirmed that Černovír has significantly worse humidity conditions than in Hrdibořice. For the success of repatriation work, the selection of micro-plots with higher soil moisture at localities is therefore important every year.

It is necessary to carry out detailed research on possible places where the introduction of the plant could be made, which would increase the stability of its population.

Experiences in the Czech Republic: Planting should take place in autumn, preferably in September-October, so that the seedlings have time to take root before the onset of frost and do not freeze. New plots should be targeted using GPS and plotted on maps. It will only be appropriate to implement the plantings after the condition of the meadows on the site has been improved. Because one of the main factors limiting the renewal of the Angelica population is precisely the very poor quality of the moisture-preferred meadows

4. Monitoring of the impact of interventions

The goal of monitoring the state of European-significant phenomena is to fulfill the reporting obligation of EU member states according to Article 17 of the Habitats Directive (92/43/EEC). EU member countries are obliged to draw up an Assessment Report on the status of species at six-year intervals. This report requires up-to-date knowledge of the species' distribution, population values, population and range trends, and an assessment of the species' habitat and threats (all at the most accurate level achievable). The achieved results are also used as a basis for the care of endangered species and their habitats, both at the level of national concepts and at the local level in the case of individual monitored locations.

- Monitoring of the number of individuals in a natural population
- Monitoring the habitats of the species, neighbored species, alien and invasive species (Braun-Blanquet scale)
- Monitoring the status of Marsh Angelica populations in areas regularly maintained by mowing. and comparison with areas that have been abandoned.
- Regular monitoring of the water regime and the groundwater level continuously with the help of data loggers.
- Monitoring of the cultivated population - the number of flowering individuals and the number of sterile individuals determined by a qualified estimate.

Monitoring unit

The monitoring unit is the individual, flowering or non-flowering. Due to the monocarpy of the species, the individual is easily distinguishable, difficulties may arise only if the plants are offshoots or they create more flowering stems, e.g. due to the cutting of a flowering plant. If such a plant grows in involved higher vegetation, it is difficult to ascertain the true condition. When counting flowering plants, when there are hundreds of them on the site, this is not considered, if the distinction is not clearly visible.

Extensive monitoring of the number of individuals in the natural population

The basic monitored variables are the number of flowering and sterile plants. The number of flowering individuals is determined by adding or qualified estimate. The estimate is made for the number of more than 2,000 flowering plants on the site by counting the individuals on a part of the population density, it is advisable to choose a known population and by area approximation of the number of individuals on the rest of the site. Depending on the situation, different reference units are chosen.

The number of sterile individuals is also determined by adding or qualified estimate. Due to the frequent massive emergence of seedlings from achenes that have fallen near the mother plants and their small size, in which they can remain for a very long time, it is practically impossible to carry out an exact count of all sterile individuals. Therefore, the sum or estimate of the number of sterile individuals with a leaf length of at least 15 cm (adult seedlings) should be taken into consideration. In addition to year-on-year comparisons of population status, the data can also indicate the number of plants that might be able to flower next year. The estimate will be made by counting tens, hundreds or thousands of individuals, according to the number of individuals in a given year, or the estimate can be made by finding the average number of individuals per unit area and this number multiplying the entire occurrence area. It is possible to choose more reference areas and thus take into account areas with different densities of sterile plants. Due to the large annual fluctuation, the number of grown seedlings can be determined by a relatively rough estimate, but in such a way that in the longer term it can be seen whether the population is viable.

When monitoring localities, other data necessary for determining the overall state of the population of the species and the state of the habitat, including negative and positive influences are also recorded.

Monitoring frequency and period

Extensive monitoring should be carried out annually or at intervals of 2-3 years for more stable populations, depending on the state of the sites. The most suitable period for counting plants is the flowering period of the species, which is from July to August. Fruiting plants are well distinguishable even during August. The areas where the planting and sowing of the species are carried out and the areas with the spontaneous appearance of plants are demarcated by stakes to prevent the plants from being cut down by large-scale mechanization. The usual time for the first mowing is at the turn of May and June, the second mowing usually takes place from mid-August. The stands in the defined areas are cut once a year only after the scizocarpum have matured, so the period for counting is wide. However, for the sake of year-on-year comparability of the data, the census should be carried out in the same period, preferably in the first half of July.

Monitoring of the species habitats

The areas should be monitored according to the Braun-Blanquet methodology in the following steps:

1. a representative vegetation composition is selected,
2. the investigation area should be geocodified with coordinates on all four sides,
3. enter all parameters for the surface (size, coverage, slope, exposure),
4. according to the Braun-Blanquet methodology, an inventory of all plant species and their cover value should be made,
5. this procedure will be recommended to be repeated every 3-5 years to monitor the state of the habitat, through the floristic composition, especially the presence of characteristic species,
6. the possible increase of the number of accompanying species atypical for the examined type of vegetation, will mean its succession and transition to another type of vegetation,
7. control of the increase of invasive species.

OUTPUTS FOR PRACTIONIONERS

Q1. Are there successive processes in the meadows that Angelica grows (woody plant species, shrubs, ruderal plants, pastures elements etc.) as a result of abandoned traditional practices?

Yes: Remove the species that are not suitable and start establishing regular annual mowing (1–2 times a year depending on conditions)

No: Q2

Q2. Does the habitat meet the needs of the species in terms of water regime?

Yes: Q3

No: Measures for managing the water regime should be taken. Based on the situation, it should be assessed which water regime management technique is most appropriate for the area where the species is developing.

Q3. Is the Angelica population small and isolated?

Yes: Several measures should be taken depending on the situation: monitoring, protection of the area, planting of new individuals, ex-situ conservation etc.

No: Q4

Q4. Is the population of Angelica stable?

Yes: Q5

No: Establishing regular monitoring of the species and its habitat. Comprehensive analysis of the threats that cause its instability and appropriate measures for their prevention/mitigation

Q5. Have measures for ex-situ conservation been taken so far?

Yes: regular maintenance of the seed collection, seed germination and cultivation of the plant in order to maintain the existing genetic pool

No: Collected seed for seed banks, cultivation, in vitro culture

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GUIDELINES

for species conservation

General guidelines for *Arnica montana* conservation

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Arnica montana

General guidelines for *Arnica montana* conservation

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Introduction

Arnica montana is listed in Annex 5 of the European Union Habitat Directive 92/43/CEE of 21 May 1992 and in Annex D of the Council Regulation (EC) No 338/97 of 9 December 1996 on the protection of species of wild fauna and flora by regulating trade therein. It is declining everywhere in European lowlands (Falniowski et al. 2011, Duwe et al. 2017, Hollmann et al. 2020). Many populations at low and middle elevations are declining even in apparently suitable habitats. Nowadays, the majority of all remaining populations in the lowlands have a number of individuals that is below the critical level for the species to maintain viable populations in the long term (Luijten et al. 2002). Recruitment in small populations is mostly absent, because of the specific conditions for seed germination and the growth of young plants in the first stages of development. Vegetative reproduction in the remnant populations will likely postpone extinction, but it is essentially an evolutionary dead end (Luijten et al. 2000).

The causes of its endangerment are multifactorial, starting with the disappearance or modification of habitats, climate change, but also the not adequate and excessive professional harvesting of flower heads for medicinal purposes. Without suitable management measures, populations will continue to decrease in size and lose genetic diversity.

Arnica montana

SCIENTIFIC PART

1. General information about the species

1.1. Taxonomy

1.1.1. Nomenclature

Two subspecies were described (Schmiderer et al. 2018): *Arnica montana L. subsp. atlantica* and *Arnica montana L.* subsp*. montana.*

1.1.2. Life form

Arnica montana is a rosette-forming perennial forb (hemicryptophyte). The species can live several decades. Due to the strong root system, it is easily propagated vegetatively by its branching, and artificial clonal propagation by parts of the root is also easy. A population can therefore consist of a network of numerous ramets belonging to only a few genets (Luijten et al. 1996).

1.1.3. Variability

There are morphological differences in the two subspecies. Compared to *A. montana subsp. montana, A. montana subsp. atlantica* is much more clonal, has smaller height, thinner floral stems, lanceolate leaves and smaller flower heads with fewer bracts (Schmiderer et al. 2018).

Within the same population, there can be great variability in reproductive traits, e.g. the number of flower stalks per plant can range between 1 and 27 and the number of flower heads per flower stalk between 1 and 13. Depending on the type of reproduction, the quantity of seeds produced per plant can be very variable: on average 20 (selfing), 40 (intrapopulation crossing) or 60 (interpopulation crossing) (Luijten et al; 2002). Seed weight is lower in small populations, and there is a significant positive correlation between seed weight and germination percentage.

The number of flowering ramets per genet can vary between 1 and 25 depending on the age of the plant and its mode of establishment (clone seedlings, rosettes or by sowing) (Sugier et al. 2013). In addition, the richness of nutrients in the soil and water supply also play a significant role in the variability of the species.

Large differences also exist in the active substances contained in the flower heads, e.g. sesquiterpene lactones, phenolic acids and flavonoids (Jurkiewicz et al. 2010).

1.1.4. Karyology

- According to Klotz et al. (2002):
- Chromosome number: 2n= 36 or 38
- Ploidy level: diploid
- Ploidy type: palaeopolyploid

1.1.5. Genetics and genomics

Most genetic variation is found within populations rather than among populations. There is hardly any genetic exchange among populations and therefore a high genetic differentiation among populations (Duwe et al. 2017).
Genetic diversity in lowland populations is lower than in mountain populations (Duwe et al. 2017). A study of 8 pop-

ulations in Belgium and Northern France showed a significantly positive correlation between flowering population size and multilocus genetic diversity, allelic richness and Wright's inbreeding coefficient at the ramet level (Van Rossum & Raspé 2018).

Mountain populations in the Alps seem genetically unaffected from genetic erosion despite strong genetic differentiation due to high genetic diversity and no inbreeding (Duwe et al. 2017). In lowland populations, there is however some evidence of genetic erosion and a tendency for increased clonal reproduction (Duwe et al. 2017). Many of these lowland populations are also very small and suffer from reduced seed quality (Godefroid et al. 2016). It is suggested that lowland populations depend on human intervention such as assisted gene flow and population reinforcement to avoid further genetic erosion (Duwe et al. 2017).

There is a significant positive correlation between population size and the expected heterozygosity (He) and the number of effective alleles (Ne) (Van Rossum et al. 2020).

Limited seed dispersal is responsible for the strong isolation by distance pattern (Maurice et al. 2016), but changing environmental conditions also play a role in this pattern.

Microsatellite markers have been developed to facilitate conservation genetic applications (see Duwe et al. 2015, Van Rossum et al. 2020).

1.1.6. Hybridization

In Europe, only two native species exist (A. montana and A. angustifolia) and they only co-exist in the north of the continent. No hybrids between these two species have been reported.

1.2. Species distribution and conservation status

1.2.1. Species area

The species is a European endemic. Its core distribution range is in Central and Eastern Europe, but it is native to following countries (POWO 2024), i.e. Austria, Baltic States, Belarus, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, Finland, France, Germany, Great Britain, Hungary, Iceland, Ireland, Italy, Montenegro, Netherlands, North European Russia, Northwest European Russia, Norway, North Macedonia, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Ukraine.

Fig. 1. A detailed distribution map.

The subspecies montana is present throughout the species' range except in Portugal, while the subspecies atlantica is present from southwest France to southern Portugal (Schmiderer et al. 2018).

1.2.2. Occurrence in particular countries

Arnica montana is red-listed in many European countries, e.g. in Belgium (EN); Bosnia and Herzegovina (VU), Czech Republic (VU); Germany (EN); Hungary (EW); Luxembourg (CR); Netherlands (EN); Norway (EN); Portugal (NT); Romania (VU); Spain (NT); Sweden (NT).

1.3. Biology and ecology

1.3.1. Phenology

Flowering in June/July, fruiting in August/September, germinating in autumn or spring. On the high mountains of the Dinarid, flowering of A. montana takes place later (from July to the second half of August).

1.3.2. Life strategy

S-stress tolerator.

1.3.3. Reproduction

1.3.3.1. Generative

- Reproductive system: allogamous, self-incompatible
- The proportion of flowering plants, the number of flowering stems and flowerheads produced per plant and seed set increase with population size
- Dicliny: gynomonoecious
- Dichogamy: protandrous
- Age at first flowering: 1-3 years
- Soil seed bank: transient

1.3.3.2. Vegetative

Clonality: lateral spread is 0.01-0.25 m/yr (Klimešová et al. 2017).
A mature plant has several rosettes connected by rhizomes (Kahmen & Poschlod 2000). Clonal propagation is predominant (due to the high sporophytic self-incompatibility of the species, Van Rossum et al. 2000). The species sometimes forms very dense mats, especially on deep and moist soils. A single plant can produce several new stems by rhizomes each year. The high clonality of the lowland populations can lead to an overestimation of the genet numbers (Luijten et al. 2000), so that the threat level may be more critical than it appears on the field (Van Rossum & Raspé 2018).

Seedling size is positively correlated with population size (Luijten et al. 2020).

1.3.4. Plant life cycle

Seeds germinate rapidly (start within 7 days; 50-70% germination after 14 days). There is a positive correlation between seed weight and the likelihood of germination as well as between seed weight and the quality of seedlings.

One-year-old plants are already capable of flowering, but produce a small number of flower stems. A plant can live for several decades.

1.3.5. Ecology requirements

The species is characteristic of nutrient-poor grasslands (e.g. Nardus grasslands) and dry heathlands, on oligotrophic siliceous soils (pH: 4.5-6.0). The species' environmental optimum is at montane elevations where summer aridity is reduced (Stanik et al. 2020).

Phytosociological alliances: *Nardion strictae, Rhyncosporion albae, Daboecion cantabricae, Trichophorenion germanici, Polygono-Trisetion, Genisto pilosa-Vaccinion uliginosi*

EUNIS habitat type : code E4.3161, E4.314, E1.712.

Ellenberg ecological indicator values: L: 9; T: 4; F: 5; R: 3, N: 2
It can occur both in dry or wet areas, but soil water saturation during the growing season negatively affects the survival of the species, because it does not tolerate stagnant water in the soil.

1.3.6. Biotic factors

1.3.6.1. Herbivory
The species is often attacked by slugs. The impact of slug herbivory on *A. montana* appears to increase with decreasing altitude. In Germany, damage from molluscs has been reported to be negligible at 610 m a.s.l., low at 385 m a.s.l. (with 8% of Arnica leaves removed) and severe at 180 m a.s.l. (75% removed). Spring is the most vulnerable time (when the young leaves emerge). Slugs also selectively graze on seedlings (Bruelheide & Scheidel 1999).

1.3.6.2. Pollination
Arnica montana is pollinated by Bees, Diptera (Syrphidae), Lepidoptera, Coleoptera (Knuth 1908, Kahmen & Poschlod 2000).

1.3.6.3. Symbiosis
Arnica montana is always associated with arbuscular mycorrhizal fungi (Jurkiewicz et al. 2010, Ryszka et al. 2010). The following symbionts have been found to be associated with Arnica root samples: Glomus geosporum, G. constrictum, G. intraradices, G. mosseae.

1.3.6.4. Antagonism
The larvae of the fly Tephritis arnicae feed on tubular flowers (Ellis 2021). The larvae of the diptera Melagromyza arnicarum live and feed in Arnica stems and transform either in the stems or in the rhizomes (Ellis 2021).

The caterpillar of the Lepidopteran Digitivalva arnicella is also exclusively associated with Arnica montana. It feeds on leaves in which it digs galleries (Ellis 2021), and lays eggs from which larvae will develop.

Entyloma arnicale is a species of plant-parasitic fungus that causes rusty-brown spots to appear on leaves (Ellis 2021).

1.4. Species threats

1.4.1. Current
Eutrophication is recognized as the main threat to *A. montana* (e.g. Maurice et al. 2012, Hollmann et al. 2020). In addition to increasing competition, eutrophication is also responsible for the disappearance of the mycorrhizae that are necessary for the development of *A. montana*.

Other environmental factors that are particularly detrimental to the conservation of *A. montana* are soil acidification (Fennema 1992) and abandonment of agro-pastoral practices (*A. montana* does not resist the competition of heathland-dominating chamaephytes such as Calluna vulgaris and Vaccinium myrtillus).

Arnica montana is one of the most important medicinal plants (containing over 150 biologically active substances) and its exploitation for medical purposes contributes to the decrease of the species. There were 250 tons of fresh flower heads harvested in Europe in 2005, with three main supplier countries: Spain, France and Romania. A reduction in the number of flowering plants has been observed if we pick >50% of the population (in fact in Romania 50% is the picking threshold adopted) (Christelle Jager, pers. comm.). The alternative would be industrial production, but large-scale cultivation is difficult and non-profitable (low yields with insufficient income for a sustainable production, Radanović et al. 2007). It is sometimes replaced by Arnica chamissonis var. foliosa (an American species), but its chemical properties are not identical to those of A. montana, so. A. chamissonis var. foliosa is not accepted in all countries for medical purposes or its use in the cosmetic industry.

1.4.2. Future

Environmental conditions will become less suitable under future climate change. Long dry periods after seed shed reduce their vitality and germination capacity. Low snow cover and dry spring are unfavorable for flowering. Juvenile plants are sensitive to moderate drought, and summer aridity is also an important climate-related predictor for plant fitness (better performances with lower summer aridity, Stanik et al. 2021). Growth and flowering are negatively impacted by increasing temperatures, indicating a higher risk of extinction of marginal populations and therefore a probable contraction of the range of *A. montana* as climate change intensifies (Vikane et al. 2019). The limited altitude of lowland populations does not allow the species to migrate upwards to compensate for future conditions less suited to its ecological requirements. There is also the problem of natural succession of competitive species, as well as the increasing occurrence of invasive species. In the current context of climate change, the species seems to have found refuge in forest edges or sparse forest environments (empirical observations on certain sites).

Ageing of many remaining populations due to the lack of generative reproduction is a current issue whose consequences will be dramatic in the future (Maurice et al. 2012).

1.5. Previous implemented management interventions

1.5.1. In-situ

In situ conservation actions are undertaken in several European countries, e.g. removal of competing native vegetation or invasive taxa, habitat protection, fence construction, translocations and public information. For instance, there have so far been 63 translocations (reintroductions or population reinforcements) implemented mainly in Germany, Luxembourg, Belgium and the Netherlands, using plug plants grown from wild-collected seeds. In two thirds of the cases where the results were communicated, there were less than 50 plants surviving at the last monitoring date. Drought, weed competition, predation and lack of management were the most frequently cited reasons for failure.

1.5.2. Ex-situ

Seed banking

Arnica montana has orthodox (desiccation-tolerant) seeds (Fig 1). They can therefore be stored in dry-cold conditions while maintaining their viability for decades. A total of 573 accessions (representing at least 640,000 seeds) have been reported to be stored according to FAO standards in European seed banks in 13 countries (Austria, Belgium, France, Germany, Italy, Lithuania, Luxembourg, Norway, Poland, Slovakia, Slovenia, Spain, United Kingdom).

Fig. 1. Arnica montana seeds with (left) or without (right) pappus. Photos Maarten Strack van Schijndel

Multiplication

Its propagation is notoriously difficult and seems to work only in greenhouses and under certain conditions. If planted in full ground, the plant is strongly attacked by insects and molluscs. As the species has been found to be always mycorrhizal on the field (Jurkiewicz et al. 2010), it is beneficial to inoculate mycorrhizae in the growing soil (Glomus group A) to avoid chlorotic plants (Fig. 2). These allow better growth and survival of cultivated plants and help them settle into the environment where they will be transplanted.

Fig. 2. 18-week-old plants showing chlorosis when using a potting soil without adding mycorrhizae. Photo Sandrine Godefroid

2. Aims of the conservation guidelines

The objective of these conservation guidelines is to provide practitioners with as much information as possible to enable them to implement measures to restore *Arnica montana* populations.

For this, key information has been brought together in section 1 of this document in order to understand the biology and ecology of the species. Then, in section 3, different interventions are suggested to help with the conservation of the species.

3. Suggestion of interventions

3.1. Site management

Maintaining or restoring traditional land use practices is the common point of any effective conservation approach (e.g. Mardari et al. 2019), as well as a regulated system of sustainable use. The different possible options are detailed below.

3.1.1. Tree cutting

Woody species exert enormous competition on *A. montana* by increasing shade, litter accumulation, and water uptake. They must therefore be eliminated as soon as they appear (e.g. *Betula pendula, Picea abies, Pinus spp, Salix* spp.) together with the accompanying plants (e.g. *Pteridium aquilinum, Rubus sp., Cytisus scoparius, Calluna vulgaris, Vaccinium* spp.).

3.1.2. Mowing

Mowing with hay removal reduces nutrient supply, decreases competition, improves light availability at ground level and prevents litter accumulation. All this facilitates seedling establishment (Hollmann et al. 2020). Germination and seedling establishment will be higher where the vegetation is not cut too short when mowing (which creates a humid microclimate preventing the soil from drying out).
In the Netherlands, mowing in September/October once every 2-3 years works very well (Gerard Oostermeijer, pers. comm.).

3.1.3. Burning

Fire is sometimes criticized by managers because of its possible impact on soil fauna. However, when applied at the end of winter, it will not have a significant impact on soil invertebrate communities (René Dahmen, pers. comm.).

Prescribed burning is also often difficult to implement due to legal restrictions or the reluctance of the local population. In areas under the control of military authorities, management by fire however allows the development of significant populations of *Arnica montana*, which attests to the pyrotolerant nature of the species. In palynological studies, when pollen of A. *montana* is found, it is always associated with the presence of charcoal, so in ancient times the species already seemed favored by fire (Gerard Oostermeijer, pers. comm.). However, to maintain the density of the plants and prevent competitive species (e.g. *Calluna vulgaris*) from re-invading managed sites, it is necessary to burn on a recurring basis (Wittig et al. 2020).

3.1.4. Grazing

Grazing creates open microsites that are essential for fostering reproduction and rejuvenation of the populations. A minimum intensity of grazing after achene maturation can therefore be considered to maintain populations and reduce competition, but excessive grazing negatively affects A. *montana*.

Grazing also improves diaspore dispersal as Arnica seeds are easily carried in the fur of animals, which greatly improves recolonization of former habitats and gene exchange between populations if there is a rotation of the herd between sites. Without the help of animals, passive seed dispersal is limited to a few meters around the mother plant (Luijten et al 1996, Strykstra et al. 1998), since dispersal by wind is very ineffective (heavy seed and poorly developed pappus).

3.1.5. Removal of topsoil layer

Removal of top soil layer has several advantages in degraded environments: (1) it removes the upper layer in which the phosphorus is concentrated; (2) it reduces the cover of undesirable competitive species; (3) it creates a bare soil that is beneficial for both vegetative (if the root is not removed) and generative reproduction of A. *montana*. However, care must be taken to ensure that only the upper part of the soil (the organic layer) is removed, in order to avoid Al toxicity effects that may result in decreased germination and establishment of A. *montana* (van den Berg et al. 2003).

When the plant composition is not too degraded (i.e. species characteristic of the targeted habitat are still present), top soil removal is however not recommended as it contains 90% of the seeds of heathland species. In Dutch heathlands, shallow turf cutting has been found to be a better technique to promote germination and establishment than sod cutting down to the mineral soil (van den Berg et al. 2003).

If the vegetation in place is not too degraded, raking is also a technique that works very well on the vegetative reproduction of A. *montana* (promotes the appearance of new rosettes, e.g. Streitberger et al. 2022). The effect of this technique is, however, less lasting over time compared to a complete removal of the topsoil (the herb and moss layers can already return to their initial level after 2 years). When raking is applied in an existing population, special care needs to be taken in order not to uproot the plants (Streitberger et al. 2022).

3.1.6. Fencing

As A. *montana* is not significantly impacted by ungulates, there is no need to fence off existing populations. However, during translocations of ex situ multiplied plants, it may be useful to temporarily fence the planting area so that the young plants have time to: (1) strengthen their root system (so as not to be uprooted by grazers); (2) develop a more robust rosette, and (3) flower and disperse seeds into the immediate environment. In order to avoid the development of overly competitive vegetation, it is important that these plantation areas are integrated as soon as possible into the normal management regime of the habitat, which implies that the fence must be removed as quickly as possible (ideally from the second year).

3.1.7. Reduction of flower harvesting

Picking recommendations for professionals: only collect from dense sites (> 5 flowering plants/m^2); only one harvest per site and per year, taking a maximum of 50% of flowering plants, distribute the picking evenly and leave the picking site to rest every 4 years (Maïlys Rumeau, pers. comm.).

3.1.8. Liming

On very acidic soils, liming promotes establishment. This is due to the fact that a high Al:Ca ratio is known to inhibit germination and to reduce growth and vitality of A. *montana* (van den Berg et al. 2003).

3.2. Species targeted interventions

3.2.1. Ex-situ conservation

3.2.1.1. In-vitro culture

Arnica montana has been propagated by using different *in vitro* culture protocols. Different factors such as the genotype, explant type and plant growth regulators significantly affect this process (Petrova et al. 2012). Nodal segments and shoot tips of *in vitro* germinated seedlings or plants are the explants normally used for shoot cultures initiation. In most cases, the basal medium was MS (Murashige and Skoog 1962). Plant growth regulators are required and culture media normally included cytokinins, alone or combined with auxins. Although different mixtures have been tested, higher multiplication rates have been reported by using 6-benzylaminopurine (BAP) combined with α-naphtaleneacetic acid (NAA) or indole-3-acetic acid (IAA) (Petrova et al. 2012). *In vitro* rooting is normally carried out on MS medium at half strength supplemented with NAA or indole-3-butyric acid (IBA), although it has also been achieved in full MS medium and in culture medium lacking plant growth regulators (Petrova et al. 2012).

Using stem segments as initial explants, Petrova et al. (2011; 2021) and Nikolova et al. (2013) reported *in vitro* shoot multiplication on MS medium with 30 g l^{-1} sucrose, 1.0 mg l^{-1} BAP and 0.1 mg l^{-1} IAA. Micropropagated shoots were rooted in MS medium at half strength containing 0.5 mg l^{-1} IBA. Cultures were incubated at 22 ± 2°C under a 16-h light photoperiod (40 µmol m^{-2} s^{-1}1). Alternatively, Surmacz-Magdziak and Sugier (2012) micropropagated *Arnica montana* plants by culturing shoot tips on MS medium supplemented with 0.1 mg l^{-1} BAP and gelled with 7 g l^{-1} agar. Rooting of shoots was carried out by transference to MS medium with 0.1 mg l^{-1} NAA and 7 g l^{-1} agar, although longer roots were developed with 0.05 mg l^{-1} NAA.

In vitro culture systems also influenced in vitro shoot multiplication. Petrova et al. (2014) found that the temporary immersion system (RITA®) gave rise to higher shoot multiplication compared to solidified medium and liquid medium with the explant supported by filter paper-bridges.

Indirect shoot organogenesis has also been investigated in *Arnica montana* (Petrova et al. 2011). Young leaves and petiole segments from three-month-old *in vitro* micropropagated plants were used as explants for organogenic callus induction in MS medium with 1 g l^{-1} casein hydrolysate, 30 g l^{-1} sucrose and 0.1 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4.D) solidified with 6 g l-1 agar. Shoot regeneration was observed after transference of leaf-derived callus to MS medium supplemented with 1 mg l^{-1} BAP and 0.1 mg l^{-1} 2,4-D. Nevertheless, shoot regeneration occurred at low frequency and further optimization was required.

Culture conditions have also been optimized for *in vitro* storage by slow-growth (Petrova et al. 2021). Culturing on MS at half strength with 2% sucrose and 3% sorbitol under low irradiance (20 µmol m^{-2} s^{-1}) efficiently retarded growth of the *in vitro* cultured plantlets.

3.2.1.2. Seed banks

Arnica montana seeds maintain their viability for decades in dry-cold rooms. Seed collections in relict populations are therefore recommended in order to capture as much of the genetic variability of the taxon as possible across its distribution area.
Storage conditions are those recommended by the FAO for long-term conservation: drying at 15% RH, then freezing at -20°C in airtight containers. Glass jars, flame-sealed vials and heat-sealed tri-laminated aluminum bags represent the only truly airtight options. Any other container should be avoided, even if manufacturers claim that it is airtight (lab tests have revealed that this is not).

3.2.1.3. Plant cultivation

Plant cultivation (Fig. 3) using the following protocol works well:

Sowing

Sowing in potting soil is *not* recommended: low germination, slow and uneven growth (Godefroid et al. 2016). Sowing in controlled conditions (on agar in incubated Petri dishes) at a constant temperature of 20°C gives much better results (>80% germination after 14 days). No stratification is required (no seed dormancy).
The germinated seedlings are delicately transplanted into seedling soil in seed trays (allows better root development than transplanting directly into pots).

Potting soil

Recommended pH between 5.5 and 6.5.

An example of substrate that works very well is Klasmann Ref. 416 TS 3 fine special sowing:
https://klasmann-deilmann.com/wp-content/uploads/Plaquette_KDF_2018.pdf

Adding granulated mycorrhizae is necessary (otherwise leaf chlorosis appears). Example of a suitable product:
https://dcm-info.be/fr/pro/produits/produits-traditionnels/dcm-mycorrhizae

Repotting

When the roots are well developed in the trays, repotting (with the same potting soil) in 9 x 9 cm pots (10 cm deep) allows to have robust plants (on the other hand, with the 5 cm Jiffy pots, the survival rate after repotting is very low).

Watering

No tap water (as it may be too alkaline in some regions). Obligatory with rainwater, from below (the leaves should not be watered). Use horticultural felt so that the bottom of the pots always remains moist.

Acclimatization

Two weeks before transplanting into the field, place the plants outside to acclimatize them.

Duration of a crop cycle

Approximately 12 weeks from germination to produce plants that are ready for transplanting into the wild.

Fig. 3. Propagation of Arnica montana. Left: 2wk-old seedlings. Right: 6wk-old plants. Photos Sandrine Godefroid

3.2.2. Plant translocation (reinforcement and reintroduction)

3.2.2.1. Sowing

Sowing gives significantly worse results than planting as only a small percentage of the seeds sown produce adult individuals. When implemented in autumn, there will be a high risk that seeds are blown away or washed out by precipitation. In spring, lack of precipitation may cause seeds not to germinate immediately, thus increasing the chances of being degraded by soil fauna and fungal agents.

When seeding is implemented, the following conditions must be fulfilled in order to maximize the success rate: (1) use a very high number of seeds (>100 seeds m^2); (2) the complete removal of vegetation is necessary; (3) deep sod cutting should be avoided; (4) sowing must be carried out during periods of humid weather.

3.2.2.2. Transplanting of new individuals

Transplanting ex situ-grown plug plants is the preferable method of establishment (Fig. 4).

Fig. 4. Transplantation of 3-month-old plants in a nature reserve after soil preparation. Photo Franck Hidvégi.

Significant differences among populations for all measured fitness components suggest that reinforcement is best achieved using material from several populations (Luijten et al. 2002). In the Netherlands, after 15 years, populations introduced using mixed sources were doing much better than single-sourced populations (Philipine Vergeer, pers. comm).

In the Netherlands, the proportion of plants surviving 3 years after translocation was much higher for plants introduced as seedlings (36.92%) than for plants introduced as seeds (0.07%). However, introducing seeds stimulates rapid selection of the best adapted genotypes (Luijten et al. 2002).

In Belgium, the genetic restoration was effective already 2 years after translocation (Fig. 5), with high genetic diversity and low genetic differentiation between generations, mixing between seed source in the F1 generation and seed-based recruitment of new individuals (Van Rossum et al. 2020).

Fig. 5. Arnica montana population restored after outplanting of 500 individuals of two different origins. Left: 2-yr-old plants. Right: 5-yr-old plants (same site). Photos Sandrine Godefroid

Fig. 6. The 6-yr-old transplants have produced 186 recruits per m². Photo Sandrine Godefroid

As there is a strong genetic differentiation between lowland and montane populations, plants from montane populations might be maladapted to conditions at low altitude sites. Using seeds from montane populations for the reinforcement of small lowland populations should therefore be avoided (Maurice et al. 2016).

4. Monitoring of impact of interventions

4.1. Plant populations – genetic and biodiversity monitoring

Below we only deal with variables that are easy for anyone to measure and do not require any special equipment or budget. More details for the demographic and genetic monitoring of restored populations are presented in Godefroid & Van Rossum (2018). An example of genetic monitoring of translocated *A. montana* populations can be found in Van Rossum et al. (2020).

Population variables:

- Population size: counting all individuals in a population
- Demographic structure, i.e. number of individuals by plant life stage: seedling/juvenile/vegetative, flowering or senescent adult
- Recruitment: number of juveniles and seedlings
- Spatial extent: area occupied by the population (using a GPS)

Individual variables:

- Plant size: height of the tallest flower stem, largest diameter of the rosette
- Reproductive success: number of flower heads per stem
- Number of stems per plant
- Reproductive success: a small sample of seeds can be collected and brought back to the laboratory in order to test their germination under controlled conditions. In *Arnica montana*, seed weight can also be used as a proxy for germination capacity.

4.2. Site quality

Site quality will be monitored by recording biotic variables as well as abiotic variables, more specifically:

- Composition of the plant community: species richness and cover abundance of each species
- Disturbances to the site, e.g. management regime, grazing pressure (to be recorded with the date and period of occurrence)
- Soil: humidity can be recorded using hygrometers; fluctuations of the water table can be measured using piezometers
- Microclimate: *Arnica montana* is very sensitive to climatic variations. Temperature and relative humidity can be monitored over a period of time using data loggers placed in the restored population

OUTPUTS FOR PRACTIONIONERS

Q1. Are shrubs and/or trees present in the population
(e.g. *Betula pendula, Picea abies, Pinus* spp., *Salix* spp.)?
Yes : Remove all shrubs and/or trees
No : go to Q2

Q2. Are there competitive sp. present in the herb layer with a total cover of more than 25 %
(e.g. grasses, *Calluna vulgaris, Pteridium aquilinum, Cytisus scoparius, Rubus spp.*, etc.) ?
Yes : Remove these competitive species
No : go to Q3

Q3. Is there recruitment in the population ?
Yes : go to Q4
No : Create patches of bare soil to allow seed germination, e.g. by raking or top soil removal.

Q4. Does the population contain at least 200 flowering individuals ?
Yes : Population should be maintained by recurrent management actions, e.g. mowing at the end of the summer or burning at the end of the winter once every 2–3 years, or by sheep grazing.
No : Consider reinforcing the population using multi-source material from dynamic populations in similar habitats in the same phytogeographical region. Go to Q5.

Q5. Is seed material available in large quantities ?
Yes : Sowing may be considered, only at a high seed density (>100 seeds/m^2) and on bare soil patches.
No : Outplanting plants that have been ex situ-propagated for a short period is the only option. A 3-month cultivation on nutrient-poor soil enriched with mycorrhizae (*Glomus* spp.) produces plants ready to be outplanted (diameter ~12cm)

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GUIDELINES

for species conservation

General guidelines for *Dianthus gratianopolitanus* conservation

Elke Zippel, Carolina Sánchez-Romero

Dianthus gratianopolitanus

General guidelines for *Dianthus gratianopolitanus* conservation

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Introduction

Dianthus gratianopolitanus is a European species that occurs from France to Poland and southern England to Ukraine. The species occurs scattered from the lowlands to the mountain zone up to altitudes of 1600 m (Switzerland) and 2200 m (France). The habitats vary depending on the area of distribution, but are generally characterized by nutrient-poor, mostly base-rich locations on rocks or sandy soils. Most recent populations are small, sometimes comprising only a handful of individuals. The species has declined sharply across Europe in recent years, so that it is now considered globally as "near threat" (NT) (Onyshchenko 2022).

Dianthus gratianopolitanus is an evergreen, perennial, and cushion-forming carnation with fragrant, pink to light pink flowers that can vary considerably. Beside the potential to produce hybrids with other *Dianthus species*, the robustness and simpleness of cultivation that may be this feature the reason for the popularity as ornamental garden plant as well as for ornamental plant breeding.

All these characters, the distribution as the rareness in connection with the threat due to changes in land use, and the use as garden plant which can lead to destroy genetic integrity of wild populations popularity, make *Dianthus gratianopolitanus* to a model species to act jointly for the protection of this beautiful species across its European distribution. In Poland as well in Germany first attempts to enlarge small populations or establish new started in the last years. Species conservationists can benefit from these experiments to and measures that lead to success, modified where necessary, and to avoid mistakes.

These guidelines provide an overview of the distribution, biology and ecology of the species, examples of conservation measures for this species and recommendations on how it can be protected both in situ and ex situ. The guidelines with its recommendations are a snapshot of the current state of knowledge and experience of the authors, but do not claim to be exhaustive. Therefore – before taking action, get in touch with locals who know the sites and with experts who know the species!

Dianthus gratianopolitanus

SCIENTIFIC PART

1. General information about the species

Dianthus gratianopolitanus is a small perennial of 10–20 cm that grows in more or less dense cushions. In shady locations, the internodes are elongated so that the plants lose their cushion growth and form loose mats or turfs. The glabrous, linear and flat grey-blue-green leaves are 2–6 cm long and 1–3 mm wide and rich in sclerenchymatous tissue. Cushion growth, wax maturation of the leaves and the thick cuticle are adaptations to warm and dry as well as cold (frost-dry) conditions. The flowers, usually solitary, are borne on 10–15 cm long hairless flower stalks and the calyx is 12–16 mm long, 2–3 times longer than the calyx scales. The light to dark pink flowers are usually solitary.

The species is regarded as a glacial relict. After the melting of the glaciers, there were ideal growing conditions in the open landscape and later in the sparse and grazed forests. With increasing forestation, the species was reduced to today's relict sites (Ehrhardt 1990, Banzhaf et al. 2009).

Fig. 1 Habitus of *Dianthus gratianopolitanus*

1.1. Taxonomy

Dianthus gratianopolitanus is a vascular plant, belongs to the order Caryophyllales, the family Caryophyllaceae, the subfamily Caryophylloideae and the tribus Caryophylleae.

Based on molecular studies based on the plastid genome, the species appears to be non-monophyletic despite morphological uniformity. Individuals from geographically different populations in Central Europe can be resolved into different sub-lineages of the *Dianthus* core group (Koch et al. 2021). Plastid sequences and nuclear AFLP'S as well as genome size and chromosome number indicate that some French populations belong to a different taxon (Koch & Michling 2016, Fassou et al. 2022).

1.1.1. Nomenclature

Dianthus gratianopolitanus has a few synonyms, of which only one, *Dianthus caesius,* is common in older literature. There were a few infraspecific taxa which are integrated in recent literature in the nominate form (Fassou et al. 2022):

Dianthus gratianopolitanus Vill., Hist. Pl. Dauphiné 3: 598. 1789. Sec. Marhold (2011)
= *Dianthus caesius* Sm., Engl. Bot. [1]: t. 62. 1792 syn. sec. Marhold (2011) Ξ Silene caesia (Sm.) E.H.L.Krause, Deutschl. Fl. Abbild., ed. 2, 5: 112. 1901, nom. illeg.
= *Dianthus caesius* subsp. *adscendens* Gaudin, Fl. Helv. 3: 158. 1828 syn. sec. Kew POWO (2024)
= *Dianthus caesius* subsp. *montanus* Gaudin, Fl. Helv. 3: 159. 1828 syn. sec. Kew POWO (2024)
= *Dianthus caesius* var. *nanus* Gaudin, Fl. Helv. 3: 159. 1828 syn. sec. Kew POWO (2024)
= *Dianthus* flaccidus Fieber in Flora 17: 633. 1834 syn. sec. WFO (2018)

In the languages of Europe *Dianthus gratianopolitanus* is called hvozdík sivý, Pfingst-Nelke, Grenobler Nelke, Cheddar Pink, Oeillet bleu, Oeillet bleuâtre, Oeillet de Grenoble, Œillet de Grenoble, Œillet bleuâtre, Œillet mignardise, Garofano di Grenoble, Goździk siny and klinček sivý.

1.1.2. Variability

There is a high variability of colour and shape of the petals as well as the leaves from individual to individual, even in a population. In some populations it is possible to distinguish the individuals by their phenotypic unique look.

Fig. 2 Variability of flowers

1.1.3. Karyology

Dianthus gratianopolitanus is a polyploid (probably autopolyploid) species. Its chromosome number are 60 (Rohweder 1934, Carolin 1957, Ritter 1972, Kovanda 1982, Lippert & Heubl 1988) or 90 (Blackburn in Tischler 1931, Gairdner in Andersson-Kottö & Gair, Rohweder 1934, Genčev 1937). The indication of the chromosome number 30 (Puch 1941) is to be checked according to Weiss & al. (2002).

1.1.4. Hybridization

As the genus *Dianthus* has long been known for its hybridization, hybrids of *Dianthus gratianopolitanus* with other species of the genus, e.g. *D. arenarius* (Gärtner 1848, Wichler 1913, Andersson-Kottö & Gairdner 1931) are known.

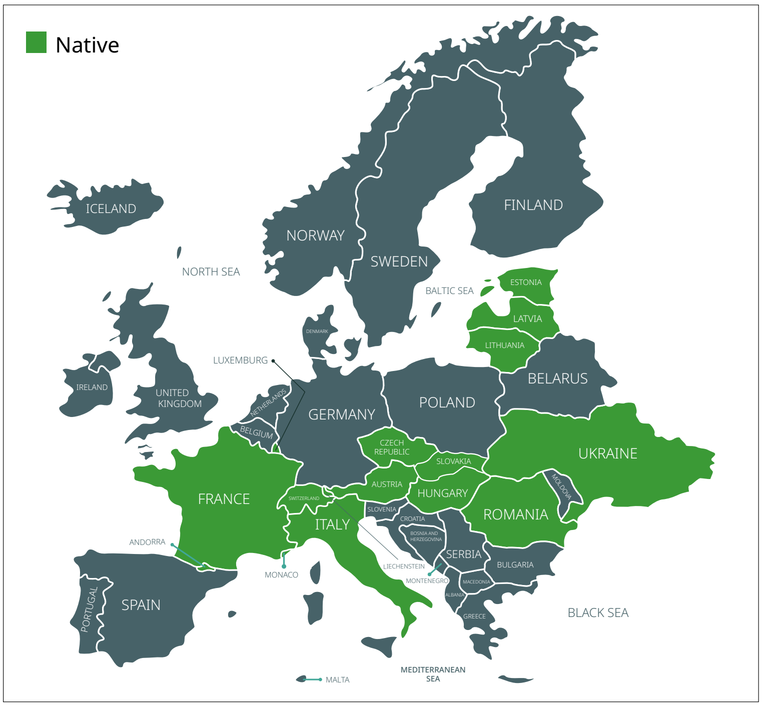
1.2. Genetics and genomics

Dianthus gratianopolitanus has so far been the subject of molecular genetic studies that used AFLPs to compare the genetic structure of populations in the Swiss and Frankonian Jura (Putz et al. 2015) and selected populations in the Swabian Jura (Koch et al. 2021).

The current molecularly detectable differentiation based on chloroplast genome sequence data of *Dianthus gratianopolitanus* goes back to interglacial Pleistocene differentiation (Koch & Michling 2016). The occurrences isolated today in the Franconian Alb show a high diversity, more than Swiss Jura populations, which are less isolated (Putz et al. 2015). In the Swabian Alb Koch et al. 2021 also showed a high degree of genetic differentiation of neighboring populations, which can be attributed to a very limited gene flow and long-term isolation and could indicate a genetic depauperation of the populations.

1.3. Species distribution and conservation status

1.3.1. Species area



Euro+Med

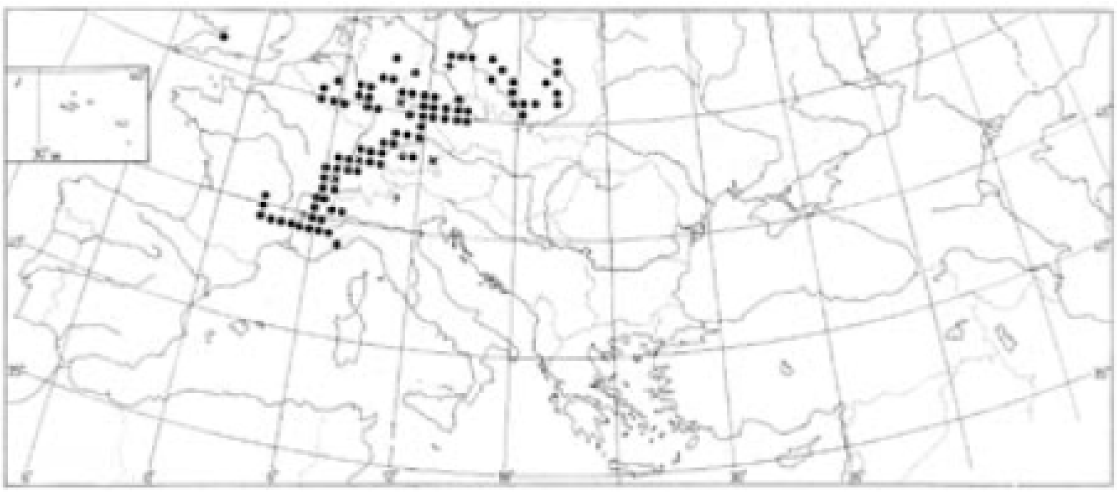
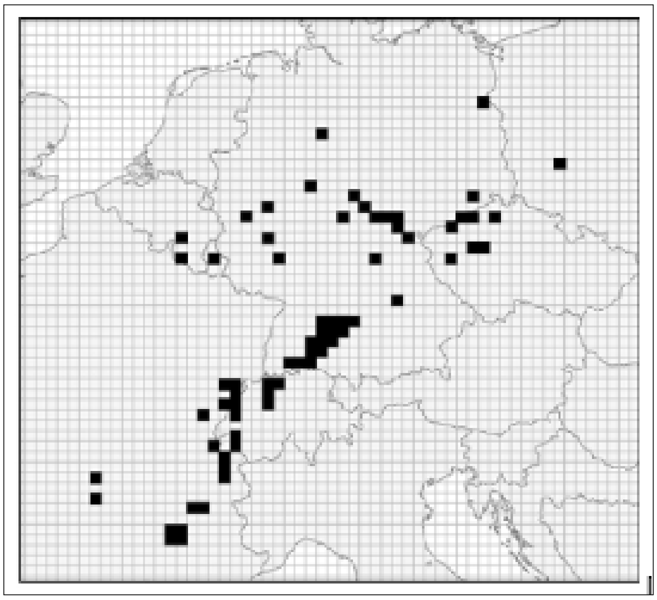


Fig. 10: European distribution of *Dianthus gratianopolitanus* Vill.. The mapping unit is the 50-km square of the UTM (Universal Transverse Mercator) grid maps of Atlas Florae Europaeae (Jalas et al. 1972). ●: native occurrence, +: extinct, ×: probably extinct, ▪ status unknown or uncertain, ? record uncertain (as to identification or locality). Reproduced from Jalas, J. & Suominen, J. (eds.), Atlas Florae Europaeae 7: Caryophyllaceae (Silenoideae), 1986, by permission of the Committee for Mapping the Flora of Europe, and Societas Biologica Fennica Vanamo.

Colling (2005)

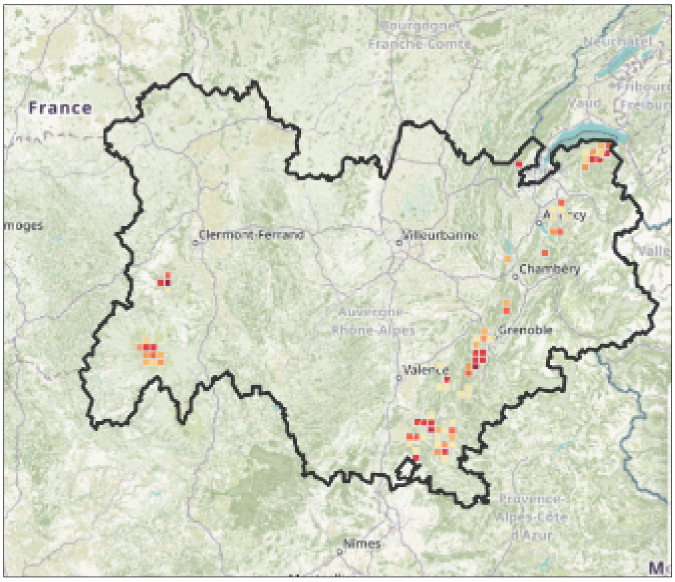


Distribution in Central Europe (Koch et al. 2021) ➞ Hier fehlen Lausitzer und polnische(?) Vorkommen.

Distribution of *Dianthus gratianopolitanus* in Great Britain and Ireland.

Stroh & Crouch 2016

https://inpn.mnhn.fr/espece/cd_nom/94756/tab/carte



https://atlas.biodiversite-auvergne-rhone-alpes.fr/es-pece/94756

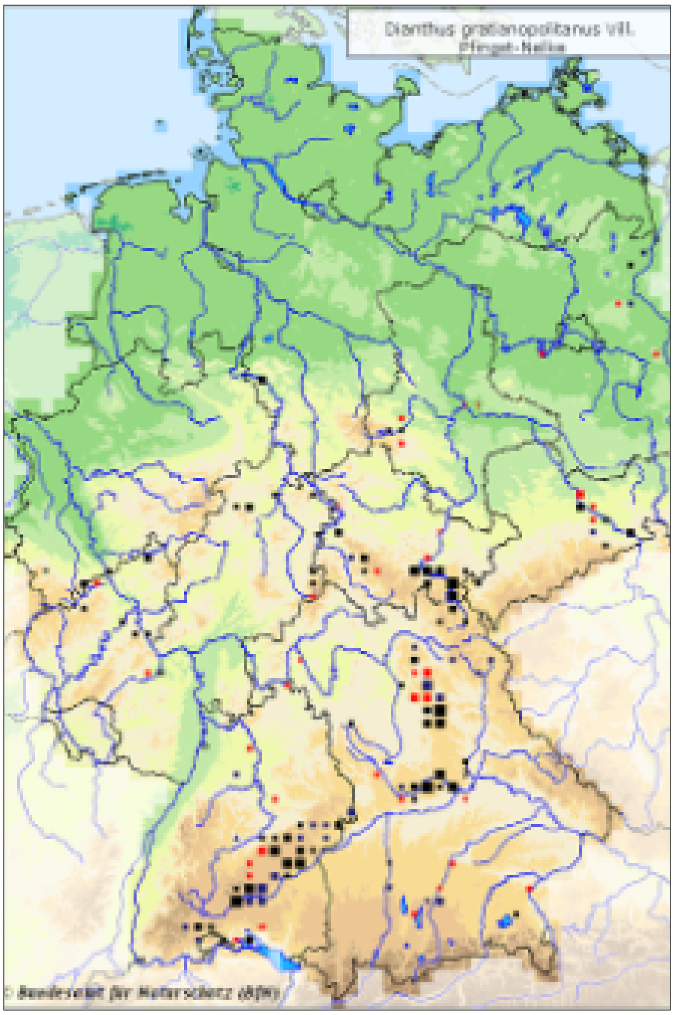


Fig. 3: Worldwide distribution of *Dianthus gratianopolitanus* map according to Colling (2005), Koch et al. (2021), Stroh & Crouch (2016), https://atlas.biodiversite-auvergne-rhone-alpes.fr/espece/94756, https://inpn.mnhn.fr/espece/cd_nom/94756/tab/carte ans https://www.floraweb.de/webkarten/karte.html?taxnr=1934

Dianthus gratianopolitanus is regarded as suboceanic element of the temperate zone of Europe (Meusel & Mühlberg 1971–1978), praealpic subatlantic / submediterrean element (Garcke 1972) or subatlantic-hercynian-central European element (Oberdorfer 1990). The distribution area comprises western and northern parts of the Alps, mainly the western French prealps, the French, the Swiss and the Swabian Jura, thinning to the east in the Frankonian Jura. The highly fragmented range of the species extends southwestwards to France in the Massif Central, while in eastern Central Europe *Dianthus gratianopolitanus* occurs from the low mountain ranges of central Germany to western Poland and Bohemia. Outposts are found in Wallonia (Belgium) and southern England (Meusel et al. 1978, DGARNE 2010, Stroh & Crouch 2016). Since long times the species is used as an ornamental plant in cottage gardens, some occurrences especially near castle ruins in the Swiss Jura (Käsermann 1999) and the Franconian Jura (Vollrath 1960) may remnants of the garden activities. It has been introduced as an ornamental plant to some other European countries (Austria, Slowakia) and can establish spontaneously, which is known from the Czech Republik (Kolbek 1975, Kovanda 1990) and England (Stroh & Crouch 2016).

1.3.2. Occurrence, conservation status and threat in particular countries

As a weak competitor and mostly chasmophytic species *Dianthus gratianopolitanus* is rare and in most countries endangered. In particular the populations in the lowlands have died out or declined sharply in recent decades and often consist of only a few individuals.

The protection status, the threat and most important occurrences in the single countries are briefly summarised below.

Belgium: CR.

A few recent populations known in Wallonia: Noue de Waulsort (Hastiére), Château-fort de Bouillon (Commune de Bouillon), Thier Pirard (Commune de Comblain-au-Pont), Les Tartines (Commune de Comblain-au-Pont). (DGARNE 2010)

Czech Republic: EN.

Rare in Western Bohemia between 220-250 m (Hradiště u Závisti) and max 688m (Lipská hora). In Eastern Bohemia along the line Úhošť – České středohoří – Bezděz – Štěchovice – Bohemian Karst – Těchoděly (Kovanda 1990).

England: VU, protected. (Cheffings & Farrell 2005)

Very rare, fewer than 5 populations. Mendip Hills of north Somerset, largest population in Cheddar Gorge (Cheffings &Farrell 2005, Stroh & Crouch 2016, UKWildlife 2016).

France: LC. Protected in the regions Franche Comté and Rhône-Alpes.

(https://inpn.mnhn.fr/espece/cd_nom/94756/tab/statut)

Grand Est. Alsace, extinct (Vangendt 2014);

Bourgogne-Franche-Comté. Franche Comté (Ferrez 2014); Ain: Reculet to the Col de la Faucille (Ain) (Käsermann 1999, Ferrez et al. 2004); Doubs: e.g.Mouthier-Hautepierre, Renédale, Lods (Doubs) (Cretin 2006); Haute Savoie: Bellevaux, La Baume, Bernex, Vailly, Vacheresse, Massif de la Dent d'Oche, Bornes (Käsermann 1999). Auvergne-Rhône-Alpes: Mont Trélod (Savoie) (Delahaye & Prunier 2006); Massif du Vercors (Isére, Drôme) to the forest of Saoû, montagne d'Angèle and Montagne de la Lance (Drôme); Massif Central: Puy de Chambourguet (Puy-de-Dôme) and Monts du Cantal (Cantal), (Observatoire de la Biodiversité en Auvergne-Rhône-Alpes 2021, Largier et al. 2004).

Germany: VU. Protected.

Baden-Württemberg: Schwäbische Alb (Swabian Jura, main area in Germany), Bodenseebecken; Bayern: Fränkische Alb (Frankonian Jura), Fränkischer Wald; Brandenburg: Märkisch-Oderland, Lausitz, Hessen: Rheinisches Schiefergebirge, Rhön. Niedersachsen: Calenberger Bergland; Rheinland-Pfalz: Eifel, Nahetal, Mittelrheintal. Sachsen-Anhalt: Harz; Sachsen: Dresden; Thüringen: Thüringer Wald, Thüringer Schiefergebirge. In the past (19th century) very common in the Swabian Jura: "on many rocks and castles in the Alb from Friedingen to Heidenheim" Martens & Kemmler (1882) as well as around Bad Freienwalde (Northeastern Brandenburg: "This adornment of our flora used to be common around the city, especially on the Carnation Mountains in the Red Land" (Schulz 1916).

Luxembourg: R. Fully protected.

One population in Michelau (Oesling). Population in Stolzembourg (Oesling) extinct (Colling 2005).

Poland: EN. Strictly protected (Rozp orządzenie 2014, Kaźmierczakowa et al. 2016).

Lower and Upper Silesia, Wielkopolska Province and Małopolska Upland: 36 localities are known, only 14 are treated as confirmed recently: Lubuskie Province (Czwałga & Wasielewski 2002; Sajkiewicz 2003, 2005); Wielkopolska Province (Węglarski & Jańczyk-Węglarska 2000); Mazovia Province (Torzewski & Kazienko 2017); Łódź Province (Olaczek 2011); Opole Province (Nowak & Spałek 2002; Kozak et al. 2005); Silesia Province (Hereźniak 2002); Świętokrzyskie Province (Łazarski 2011).

Switzerland: VU. Protected species.

37 recent, mostly small populations with only 2-15 cushions, mainly in the Swiss Jura Vaud: Chasseron; Neuchâtel: Les Brenets; Le Locle, Les Loges; Jura: St. Ursanne (to be confirmed); Solothurn: Jura range between Grenchen and Lostorf; Bern: Gorges du Court (Jura), Burgdorf and Krauchthal (Mittelland); Basel-Landschaft: Nenzlingen; Aargau: Olten; Schaffhausen: Osterfingen, regarded as translocated Population (Käsermann 1999), a couple of populations have to be confirmed.

Ukraine: **EX**. (Onyshchenko 2022).

1.4. Biology and ecology

1.4.1. Plant life cycle

Dianthus gratianopolitanus is a very long-lived species with pronounced vegetative growth. The plants should be able to live for several decades (Banzhaf et al. 2009, own observation). They are fully frost-hardy, even surviving deep frosts below -15°C without any damage (own observation in Brandenburg, Germany). According to the database of the Missouri Botanical Garden it is winter hardy to USDA Zones 3-9 (Missouri Botanical Garden Plant Finder 2024).

Seedlings are rarely observed and the establishment of seedlings is even rarer (Banzhaf et al. 2009, Zippel et al. 2021a). Koch et al (2021) were unable to find seedlings at the Swabian Jura between 2015 and 2020. They attribute this to insufficient diaspore production in the existing populations ("seed rain") and insufficient germination and seedling establishment. It is a well-known fact that the rejuvenation of plant populations in dry grassland communities is generally difficult (Wilmanns & Rupp 1966, Jackel & Poschlod 2000). That seedlings can sometimes establish despite persistent spring drought is shown by observations in Brandenburg, where seedlings developed into vigorous and richly flowering individuals within a few years (Zippel et al. 2021a). It is not known whether these seedlings are the result of germination in autumn or spring. Since the winters were quite mild in the years of these observations, germination in the autumn is likely in the Brandenburg seedlings. Seedlings usually establish in a protected, slightly shaded location on bare soil (Banzhaf et al. 2009, Zippel et al. 2021a).

Fig. 4 Seedling of *Dianthus gracianopolitanus*

Dianthus gratianopolitanus is not known to mycorrhizalize (Harley & Harley 1987).

1.4.2. Life form and strategy

Dianthus gratianopolitanus is a perennial species, which can live several decades at least. It is a Chamaephyte which evergreen rosettes are growing in more or dense cushions or mats.

Fig. 5 Loose cushion of *Diathus gratianopolitanus* in a light pinus forest in Brandenburg / Germany

The species is regarded as **csr** – intermediate competitor-stress tolerator-ruderal strategist (Frank & Klotz 1990).

1.4.3. Reproduction

1.4.3.1. Generative

Flowering starts in the lowlands in May (Meusel H. & Mühlberg H. 1971–1978, Zippel et al. 2021a, Colling 2005), in higher altitude in France June-July (Le Driant 2024). Young plants can flower within a year if the water and nutrient supply is sufficient. *Dianthus gratianopolitanus* is self fertile (BiolFlor 2014).

Putz et al (2015) found increased fruit production as a result of high population density. The fruits are dry capsules, which are ripening approximately six weeks after flowering (own data). Seeds fall out when the capsule moves when touched by animals or wind.

The size of the seeds is ca. 2 x 2,5 mm (Banzhaf et al. 2009) to 2,7-3,1x1,8x2,2 mm (Kołodziejek et al. 2018). The 1000 Seed Weight is between 0,9416 and 1,1868 g, the mean seed weight is 1,07 g (Seed information database). Kołodziejek et al. (2018) indicate the 1000 Seed Weight with 0,726+-0,08 gr (Kołodziejek et al. 2018), own measures range between 0,61 and 1,0 g(mean 0,83g).

The seeds are slightly winged, therefore they are considered to be anemochorous (Banzhaf et al. 2009). In view of their weight, the flight range is probably limited. In two occurrences in Brandenburg , seedlings were always found below plants, which suggests a narrowly limited dispersal ability of the falling seeds (Zippel et al. 2021a). Banzhaf et al. (2009) also assumes a very limited long-distance dispersal potential, as is known for dry grassland species (Jackel & Poschlod 2000). Long-distance dispersal is only conceivable as random dispersal due to the dispersal of seeds by wild animals. In view of the isolated relict occurrences and the lack of biotope connections, the development of new locations through natural dispersal vectors seems quite unlikely.

Seeds germinate in the lab and garden without specific treatments rapidly (start within 6 days; 80 % germination after 14 days) and reach usually 85-100% germination rate (own data). There is no dormancy known, the seeds germinate directly after harvesting. Germination starts in light as well as in darkness and also in cold (15/5). The highest germination rates are found near 100% in light (25/15). Cold Stratification increased the germination rate, but not the percentage of germination (Kołodziejek et al. 2018).

Nothing is known about the longevity of the seed bank in the soil under natural conditions.

Fig. 6 Capsules of *Dianthus gratianopolitanus*

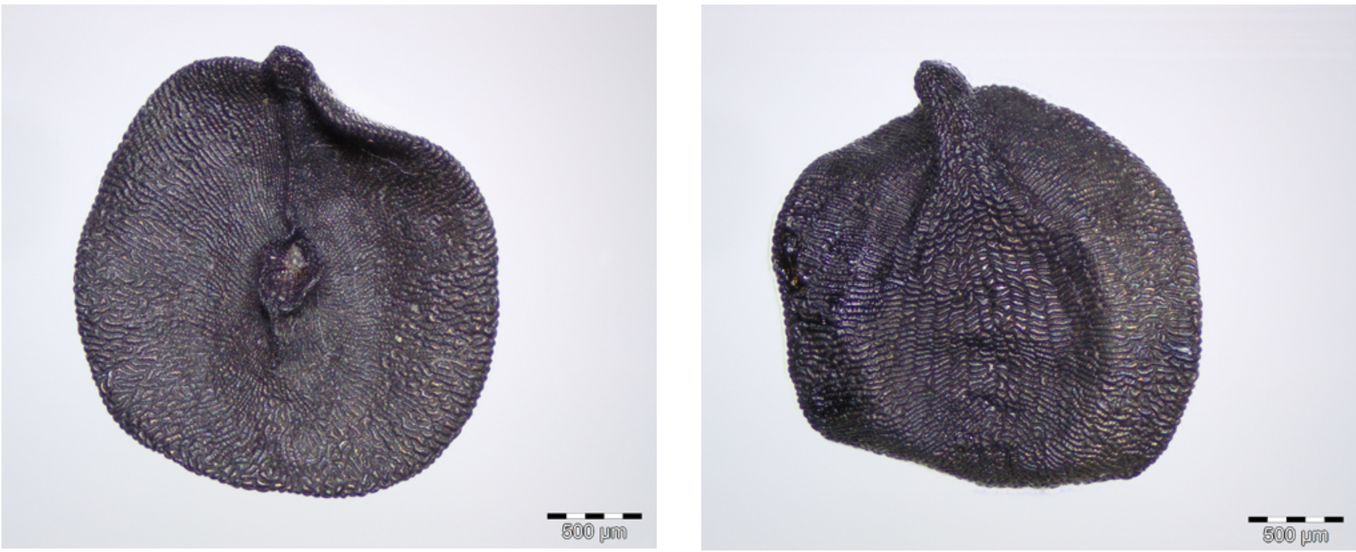


Fig. 7 Seeds of *Dianthus gratianopolitanus* (Photo: Marion Cubr, Botanic Garden and Botanical Museum Berlin)

1.4.3.2. Vegetative

Vegetative propagation plays an important role in this species. *Dianthus gratianopolitanus* forms dense cushions that can reach considerable size. The stolons are usually in the ground (Klotz et al. 2002) and can grow up to 40 cm long (Banzhaf et al. 2009). The new rosettes are formed in the leaf axils, are growing around the mother plant and can then be mistaken for seedlings. Under shady conditions, the pads thin out over time and become loose and larger mats. Populations with many plants can therefore sometimes consist of only a few clones. Clones can be distinguished in the field often by their individual flower shape and colour (Fig. 2) and sometimes also by leaf colour and shape (Fig. 8, see also Fraga et al. 2004).

Fig. 8 Different leaf shape and leaf colour of different individuals

1.4.4. Habitat

Dianthus gratianopolitanus is a species of exposed limestone and silicate rocky outcrops, which are among the few primarily forest-free sites in Central Europe outside the Alpine altitudes. However, they also owe their expansion to their former extensive use as pastureland and a source of wood.

In most regions, *Dianthus gratianopolitanus* grows on sunny cliff edges, steep rock slopes, on humus-poor inaccessible ledges and terraces, in crevices and on the thin soil of exposed rocky hill tops. These locations are mostly small-scale extreme sites where permanent succession occurs slowly or not at all due to soil erosion by wind and water, extreme daily and annually fluctuating temperature and humidity conditions.

At higher and northern altitudes of the distribution area the range, it seems to prefer south-facing locations, while in south-west Germany it shows no preference in this respect (Banzhaf et al. 2009). On strongly south-facing areas, it appears to benefit from slight protection by woods and shrubs (Witschel 1980, Banzhaf et al. 2009).

The species, with its wide and disjunct range, grows in various vegetation units whose syntaxonomic context is handled very inconsistently in the literature. However, as common phytosociological units have found their way into official classifications such as the EUNIS habitat codes, the habitats of the European Habitats Directive and other vegetation classifications, they are also listed in the following brief description of the most important habitats in which *Dianthus gratianopolitanus* occurs.

Dianthus gratianopolitanus occurs on both alkaline and acidic rocks. The main occurrences are on carboniferous limestone cliffs of French, Swiss and Swabian Jura, as well as isolated occurrences in the Franconian Jura and in the chalk formations of Wallonia and the Mendip Hills in Southern England. Here it grows in the Diantho gratianopolitani-Festucetum pallentis Gauckler 1938, which is categorised into different alliances and orders in the literature. Royer & Ferrez (2020) regard *Dianthus gratianopolitanus* as a character species as Diantho gratianopolitani-Melicion ciliatae Korneck ex J.-M. Royer 1991, in which they differentiate the Diantho gratianopolitani-Festucetum patzkei (J.-M. Royer 2011) J.-M. Royer & Ferrez 2020 as the second society in which the species is represented in the French Jura. In Bohemia, *Dianthus gratianopolitanus* is known from the Seslerio-Festucion duriusculae (Kovanda 1990). Misset (2015) classifies the communities of *Dianthus gratianopolitanus* in the Vercors as Koelerio-Phleion phleoidis and Cystopteridion.

On the south-western edge of the range, it grows on volcanic rock (Cantal, Massif Central), where it is considered as a characteristic species of Dianthion gratianopolitani Focquet 1982 (Bensettiti et al 2004). Occurrences on basalt are known from the Massif Central (Puy de Chambourguet) as well as from the Thuringian Forest and the Harz Mountains. The occurrences slat or base rich basalt like the populations in the Thuringian Schiefergebirge, the Rheinische Schiefergebirge or in the Harz Mountains are also assigned to Diantho gratianopolitani-Festucetum pallentis Gauckler 1938 (e.g., Jäger 2013). some German populations grow also on volcanic rock (Rhön, Hessen). Rarely *Dianthus gratianopolitanus* occurs in heavy-metal meadows on serpentine in the Frankonian Forest in Bavaria (Gaukler 1954).

According to Kolbek (1975) the Bohemian populations it is regarded as a character species of the Alysso saxatilis-Festucion pallentis Moravec in Holub , Hejný, Moravec et Neuhäusl 1967.

Dianthus gratianopolitanus also grows in sparse forests or edges. In Switzerland there ae some populations in sparse forest edges of the Geranio sanguinei-Peucedanetum cervariae (Kuhn 1937) Th. Müller 1961, in sparse snow heath-pine forests in stands of the Coronillo vaginalis-Pinetum sylvestris J. L. Richard 1972 or in sparse fragments of small *Quercus pubescens* forests. Well-growing, vigorous populations are known from sparse deciduous and mixed forests in Hessen (Germany) (Bönsel et al. 2018). In the north-eastern part of the distribution area (Poland, Northeastern Germany) the species grows in *Pinus silvestris* forests of the Dicrano-Pinion (Libb. 1932) Matusz. 1962 em. Schubert 1995 on mostly base-rich sandy soil (Oberdorfer 1990, Royer & Ferrez 2020).

Fig. 9: Typical habitat of Dianthus gratianopolitanus in steep silicious rock slopes and ledges in Thuringia / Germany

In Switzerland it is rarely observed in shallow, fresh grasslands (Käsermann 1999). In the Swabian Alb often rooted in moss cushions (Banzhaf 1990).

An overview of the most important habitats of *Dianthus gratianopolitanus* according to the EUNIS habitat classification is given in Table 1.

Tab. 1: List of most important habitats of *Dianthus gratianopolitanus* according to the EUNIS habitat classification

E - Grasslands and lands dominated by forbs, mosses or lichens
E1 - Dry grasslands
E1.2 - Perennial calcareous grassland and basic steppes
E1.29 - *Festuca pallens* grassland
E1.292 - Calcicline pale fescue grasslands
E1.2921 - Peri-Hercynian calcicline pale fescue
French, Swiss, Swabian, Franconian Jura, Bohemia, Wallonia, Thuringian Forest, Poland; South England
E1.293 - Acidocline pale fescue grasslands
E1.2931 - Hercynian siliceous pale fescue grasslands
Harz, Bohemia
E1.5 - Mediterranean-montane grassland
E1.51 - Mediterraneo-montane steppes
E1.511 - Mediterraneo-montane Stipa steppes
Massif de Vercors, France
G - Woodland, forest and other wooded land
G3 - Coniferous woodland
G3.4 - Pinus sylvestris woodland south of the taiga
G3.42 - Middle European Pinus sylvestris forests (acidophil or basic sand)
G3.421 - Subcontinental Scots pine forests
G3.4211 - Central European Scots pine forests
Northeastern Germany, Poland
G3.44 - Spring heath Pinus sylvestris forests (calcareous)
Swiss Jura
H - Inland unvegetated or sparsely vegetated habitats
H3 - Inland cliffs, rock pavements and outcrops
H3.1 - Acid siliceous inland cliffs
H3.11 - Middle European montane siliceous cliffs
H3.112 - Hercynio-Alpine montane and collinar siliceous cliffs
Cantal, Massif central, France
H3.113 - Hercynio-Alpine serpentine cliffs
Frakonian Forest
H3.2 - Basic and ultra-basic inland cliffs
H3.25 - Alpine and sub-mediterranean chasmophyte communities
H3.252 - Middle-European calcareous fern cliffs
Massif de Vercors, France

1.4.5. Biotic interactions

1.4.5.1. Herbivory

Probably due to the quite solid leaves as well as the saponine, which is a typical ingredient of Caryophyllaceae, seems that herbivores mostly avoid the species (Banzhaf et al. 2009). In mild winters Saum (2006, in Banzhaf et al. 2009) observed heavy mice feeding on the leaves, which could be easily and efficiently prevented by covering them with small wire baskets.

1.4.5.2. Pollination

The flowers of these carnation are typical stalked flowers: the light to dark pink petals form a narrow tube ("stalk") in the lower part of the flower, at the base of which the nectar is located. The upper part of the petals protrudes more or less at right angles to the outside and form the "disc". The lower part of the flower is enclosed by the tubular, fused calyx. The flowers exude an intense fragrance that attracts diurnal and nocturnal moths as well as butterflies. Its nectar is sucrose-dominant with a moderate sugar and a high amino acid concentration (Ehrhardt 1989). In one of the few larger populations in the Swiss Jura in an altitude of 1982 m mostly *Macroglossum stellatarum* was observed as a flower visitor, further some other diurnal hawkmoth species like *Hemaris fuciformis* as well as the nocturnal *Autographa gamma* and other moth belonging to the Noctuidae like *Hadena caesia* (Table 2). Both, shape and colour of the flowers and the flower vistors seem to indicate that the species is an intermediate form in transition to moth pollination (Ehrhardt 1989).

Sometimes ants and beetles can be observed at the flowers of *Dianthus gratianopolitanus* (own observation). Whether they can contribute to pollination by chance is unknown.

Known pollinators of *Dianthus gratianoplitanus:*

Papilio machaon, Papilionidae (Erhardt 1990)
Pieris rapae, Pieridae (Erhardt 1990)
Aglais urticae, Nymphalidae (Erhardt 1990)
Vanessa cardui, Nymphalidae (Erhardt 1990)
Sphinx pinastri, Sphingidae (BfN 2024)
Deilephila porcellus, Sphingidae (BfN 2024)
Hemaris fuciformis, Sphingidae (Erhardt 1990)
Macroglossum stellatarum, Sphingidae (Erhardt 1990)
Autographa gamma, Noctuidae (Erhardt 1990)
Cucullia umbratica, Noctuidae (BfN 2024)
Diachrysia chrysitis, Noctuidae (BfN 2024)
Euchalcia variabilis, Noctuidae (Erhardt 1990)
Hadena caesia, Noctuidae (Erhardt 1990)
Hadena compta, Noctuidae (Kephart et al. 2006)

Fig. 10 Flowers of *Dianthus gratianopolitanus*

1.4.5.3. Symbiosis

The genus *Dianthus* has a special relationship with the moth genus *Hadena* (Noctuidae), whose life cycle is dependent on *Dianthus* plants. While the males only fly to the flowers for a short time, females can be observed staying on the flowers for longer. They look for a suitable place for their eggs, which they lay between the calyx tube and the small scales of the outer calyx or directly in the flower.

The newly hatched egg caterpillars eat their way through the still tender wall of the calyx tube and enter the ovary, where they feed on the nutrient-rich ovules and the young seeds. The young caterpillars remain in the flowers or in the unripe fruit. Later, they bore their way out through a hole in the pericarp and hide on the ground during the day. Older caterpillars feed not only on the fruit but also on the leaves of their food plants and are sometimes out and about during the day. The moths survive the flower-poor midsummer as pupae or caterpillars in the ripe fruit.

This system between pollination, feeding and reproductive success is precisely balanced. With its pollination, the moth ensures a better seed set. This guarantees sufficient food for its brood, which partly destroys the seed set. The extent to which sometimes one partner benefits and sometimes the other, or whether both partners benefit from the symbiotic relationship, is the subject of numerous scientific studies and varies from case to case between antagonism and mutualism (e.g., Collin et al., 2002; Kephart et al. 2006, Prieto-Benítez 2017).

1.4.5.4. Antagonism

Beside the antagonism described above, some antagonists are known. Following pests are known from cultivated plants in gardens and greenhouses: Aphids, snails (garden); thrips, caterpillars, red spider mite (under greenhouse conditions), rust fungi on leaves and stems; viral diseases, *Fusarium* wilt caused by aphids (Brickell 2000), various viruses (Fraga et al 2004).

1.5. Species threats

1.5.1. Past and Current

Succession

Rocky outcrops as well as nutrient poor, basic and light pine forests on basic sand rare and endangered habitats, which are in decline across Europe (Welk 2002). Especial rocky outcrops with their azonal vegetation habour often a unique flora and fauna, which protection and restoration is a big challenge around the world (Fitzsimons & Michael 2017). The decline of *Dianthus gratiapolitanus* has various causes, which differ from region to region given the species' large range.

One of the main reasons for the species' decline is the succession of its habitats due to abandonment. The grazing during the last centuries had kept the habitats open and suppressed scrub encroachment. Succession is primarily due to the abandonment of sheep and goat grazing (Käsermann 1999, Banzhaf et al. 2009), but lack of timber harvesting also plays an important role in the ongoing succession (Müller et al. 2008).

Succession is also intensified by the eutrophication of the sites due to nitrogen emissions. Pollution varies from region to region but likely plays a significant role in the densely populated areas of Central Europe (Banzhaf et al. 2009), where nitrogen accumulation in the soil promotes stronger competitors. This phenomenon is described in detail by Banzhaf et al (2009) from the Swabian Alb. There, *Sesleria albicans* and *Carex humlis* benefit as stronger competitors from improved nitrogen supply and weaken the cushions of *Dianthus gratianopolitanus*. Shrubs and lianas such as *Hedera helix*, *Rosa piminellifolia, Ligustrum vulgare* (Banzhaf et al. 2009) or *Rubus*-species (Thuringia, own observation, Fig.11), which can destroy the sites of *Dianthus gratianopolitanus* within a few years, benefit equally from abandonment and nitrogen input. Nothing is known about the effect of increased nitrogen supply to a lower resistance to pathogens and herbivores due to faster growth and an associated lower concentration of secondary plant substances and less hard leaves (Kull 1991, 2001) on *Dianthus gratianopolitanus*.

Fig. 11 Dianthus gratianopolitanus is overgrown by strong competitors such as blackberries if the biotope is not cared for properly (Thuringia, Germany)

In any case, increasing shade has an influence on flower formation. With increasing shade, the number of flowers decreases, and in heavily shaded locations flowering no longer takes place (Banzhaf et al. 2009, Zippel et al. 2021a) As succession proceeds and grazing is absent, it is not only the light regime that changes. Through leaf and needle litter and emerging herbs and grasses humus and nutrient enrichment take place. Sheltered by shrubs, the layer of litter and humus is less exposed to the wind and not blown away to a greater or lesser extent, in contrast to exposed rocks (Banzhaf et al. 2009, Jäger 2013).

In some populations, there is often little or no seed set, sometimes no fruit set at all and the flower withers after flowering or falls off completely (Ehrhardt 1990, Banzhaf et al. 2009, Zippel et al. 2021a). While Ehrhardt (1990) and Banzhaf et al. (2009) rule out pollinator scarcity as a reason in view of the flower-rich habitats and the broad pollinator spectrum of *Dianthus gratianopolitanus*, pollinator scarcity may well be the case in the species-poor and raw-soil-rich pine forests, especially if the main flowering period falls in an unfavorable weather period (Zippel et al. 2021a). Inbreeding may also play a role in this phenomenon, but when cultivated under optimal, i.e. sunny conditions by even just a few individuals in the garden, abundant fruiting with good seed set can always be observed. However, quantitative studies on this are lacking.

Direct destruction of the habitat

Another reason for the decline of *Dianthus gratianopolitanus* is the direct destruction of its habitat, as can occur through mining and the expansion of quarries (BfN 2024) or renovation work on castles and ruins (Käsermann 1999) Afforestation, which destroy habitats of the species is a long-known problem, as the German botanist Schulz (1916) writes from Bad Freienwalde, Brandenburg: “This ornamental of our flora used to occur frequently in the vicinity of the city, especially on the Carnation Mountains on the Red Land. I found no trace of it there. The main cause of its destruction was probably the afforestation of the area”. Forest activities are in Brandenburg an ongoing risk (Zippel et al. 2021a).

Increasingly, outdoor recreational activities such as footsteps damage due to climbing, hiking, campfires, visiting of viewpoints (Müller at al. 2004, Saintenoy-Simon s.a., Banzhaf et al. 2009, Zippel et al. 2021a) are causing damage to habitats. The cushions on rock edges or in loose sand are very sensitive to trampling and are quickly torn out.

Considerable trampling damage can be observed, especially on rocky outcrops, which are often used as vantage points (Banzhof 1990); only in crevices where the roots are protected are the cushions less sensitive to trampling, but they do not flower (own observation Schwarzatal, Thuringia). The threat posed by climbing sports should not be underestimated in individual cases (Holzschuh 2016, Banzhaf et al. 2009). With the rise of hiking tourism and the increased use of viewpoints as picnic and campfire sites (also 1 August bonfires in Switzerland, Käsermann 1999), the protection of rock communities will become increasingly important in the future, but recreational activities will take a back seat to the lack of care and succession of the sites as a cause of endangerment.

Dianthus gratianopolitanus is a popular garden plant that used to be excavated up from wild sites populations and planted in the garden or its seeds collected for cultivation in the garden. It is also known to have been picked as a cut flower, which may have reduced or destroyed many populations. Botanists from Brandenburg are quoted here once again, who wrote very drastically more than 130 years ago: "We set off soon after 6 o'clock in the almost icy morning freshness to reach the 'Red Land' [...]. Only a few days ago, Mr. Kunow had noticed individual sticks of the 'Pfingstnelke' (*Dianthus caesius* Sm.) there, which could be found there in large numbers about a decade ago, as at various other points in the Freienwalde area. Unfortunately, it was not possible to find a single one of them again." To use the words of our trusted reporter F. Moewes, 'the tourists and the wild botanizing youth of Freienwalde are waging a relentless war against the beautiful plant as well as against the delicate feather grass, *Stipa pennata*, which will lead to the extermination of these rarities unless the authorities (as has happened in Thuringia with regard to the rare orchids) decide to take police protective measures' (Ascherson & Gürke 1890). In the meantime, excavation of the species occurs, but is only rarely observed (Banzhaf et al. 2009).

In this context, however, another cause of risk should be mentioned, the extent of which has not yet been investigated. Today *Dianthus gratianopolitanus* is sold in garden centers as seeds and potted plants. Since, as mentioned at the beginning, the genus comprises many species that can be crossed with each other and is a popular genus in ornamental plant breeding due to this fact and the high morphological diversity of the flowers, it cannot be ruled out that garden origins have been crossed with natural growth sites (Banzhaf et al. 2009, own observation).

Further, Banzhaf et al. (2009) observed damage to cushions caused by natural factors such as trampling damage and droppings on the cushions by chamois, damage caused by foraging ravens, covering of the cushions at plucking sites or winter mice feeding. As these already occurred before the current anthropogenic causes of threat, they are certainly without major importance. This also includes the pollination parasitism of the moth genus *Hadena* described in Chapter 1.4.5.

1.5.2. Future

Temperature conditions obviously play an important role in flower formation. The highest flower formation in the variety "Bath's Pink" was achieved in greenhouse trials after vernalization of several weeks between 0 and 5° C, higher temperatures led to delayed flower onset and reduced number of flowers (Padhye & Cameron 1008). Koch et al. (2021) observed earlier flower and reduced number of flowers with increasing temperature in open greenhouse cultivation.

Rising temperatures in the future are therefore likely to make it increasingly difficult for the species to successfully reproduce generatively. Due to restricted dispersal and the isolated location of potential sites, it will become increasingly difficult for the species to move to other areas.

1.6. Previous implemented management interventions

The species is regarded by the National Nature Agency as „Verantwortungsart", this means, Germany has for its conservation a high responsibility. The Federal Agency for Nature Conservation is therefore supporting the project as part of the „Bundesprogramm Biologische Vielfalt", e.g. the project „Wildpflanzenschutz in Deutschland" (WIPs-De, Zippel et al. 2016, Zippel et al. 2021b, Lauterbach et al. 2021). The project combines ex-situ conservation (seed banking, propagation and conservation in Botanical Gardens), reintroduction and population support in situ as well as education and public relations activities. The individual activities of the joint project are also listed below.

1.6.1. In-situ

Maintenance of habitats

Careful thinning of trees and shrubs showed visible success both in the Swabian Alb, in Brandenburg and Western Poland (Banzhaf et al. 2009, Borysiak et al. 2003, Jańczyk-Węglarska et al. 2013, Zippel et al. 2021a) and leads to increasing flowering of the cushions. Excessive clearing of trees and shrubs leads to a rapid and massive change in microclimatic conditions of the site. Other, more competitive species can quickly colonize places where *Dianthus gratianopolitanus* previously grew due to changes in soil conditions such as humus accumulation and eutrophication (Banzhaf et al. 2009).

Removing larger trees and shrubs in a way that is gentle on the surrounding vegetation involves considerable effort. In order to minimise the impact on the rocks and their flora and fauna, pine trees were removed by helicopter in the Kellerwald in Hessen / Germany a few years ago (NABU 2024).

In the Swabian Jura grazing showed no negative influence on the existing population (Koch et al 2021), but too intensive goat grazing can be harmful (Banzhaf et al. 2009).

Control of leisure activities

In the Swabian Jura, climbing routes have been rerouted in consultation with local sections of the Alpine clubs, redirectors have been drilled below the rock faces to prevent access to the rock faces or climbing crags have been closed. With a few exceptions, these measures can be considered successful (Banzhaf et al. 2009).

Reintroductions

Several population supports or establishings of new populations with autochthonous material has already been carried out in various regions of Germany and Poland.

Poland

In Wielkopolskie, a new population was already established in the 1990s, using material from the only remaining wild population in the region (Weglarski & Janczyk-Weglarska 2000).

In the Goździk siny w Grzybnie reserve where a drastic decline from 29 individuals with a total cover of 20 m² to only three weak individuals with an area of only 0.7 m² was observed. The population was supported in 2004 with 52 two-year-old plants flowering individuals, taken from the renewal shoots of plants in ex situ culture, were planted after the removal of *Prunus padus.* 40 vital and flowering plants were found again in 2011 (Jańczyk-Węglarska et al. 2013).

Germany

In the Lausitz (Brandenburg, Eastern Germany), population support was carried out in 2007 in the area of the still existing original population near Bademeusel using seeds and young plants from the conservation culture in the Langengrassau Heidegarten. After 2 years, both seedlings from the seeds and several of the planted individuals were (re)found (personal message A. Herrmann). In 2016, there were some single and weak plants in a very shady location, and a beech plantation had been created nearby the translocation site despite the intervention of the responsible nature conservation authority (own observation).

In the Swabian Alb, a total of 549 individuals were 2017 and 2019 planted in several areas (Koch et al. 2021). The survival rate after the first winter of the 388 individuals planted in 2017 22.8%. In 2020, 75 of the 549 individuals planted between 2017 and 2019, could still be found in 2020.

A comparatively extensive measure to support two small existing populations was carried out in Märkisch-Oderland in Brandenburg (Zippel et al. 2021a). Here, *Dianthus gratiaopolitanus* grows in very sparse pine stands on base-rich sands. On the one hand, the existing populations were supported with young plants grown from seeds or cuttings, and on the other hand, areas were sought in the immediate and wider surroundings where the establishment of a new carnation population appeared promising due to their existing vegetation and floristic composition with basiophilic species. In total, more than 2500 individuals were planted and 500 seeds sown in various areas in mostly consecutive years. The survival rate of individuals after the first winter varied extremely from plot to plot between 94,3 and 1,7%. Due to the heterogeneous conditions of these experiments, e.g. the planting in several stages over several years and the different numbers of planted individuals between 20 and 400 individuals according to different sizes of the plots, it is not possible to interpret the results of these establishments experiments uniformly. On average, around 63% of the planted individuals have survived, the established plants have grown into strong cushions, the mortality of individuals has fallen to a single-digit percentage per year and young plants are increasingly being found in some of the plots.

Fig. 12 Successful relocation of *Dianthus gratianopolitanus* in Brandenburg / Germany

However, it became clear that the establishment of the young plants depends to a large extent on the water supply of the planted individuals. Koch et al. (2021) also observed higher mortality in one of the planting areas on south and south-west exposed and thus drier and warmer planting sites.

1.6.2. Ex-situ

Seedbanking

As the seeds are orthodox (SID 2024), the storage of seeds under the usual conditions of a seed bank (dried with silica gel in airtight containers at -24°C) is recommended. It is also possible to store the seeds using cryopreservation (Arapetyan 2006). According to a survey of European Seedbanks (Ensslin et al. in prep.) eight seedbanks in Europe hold accessions of seeds of *Dianthus gratianopolitanus* (Tab.3).

Land	Seedbank	Origin of accessions	collection type	Number of accessions	number of seeds
France	Conservatoire botanique national méditerranéen de Porquerolles			1	unknown
Germany	Dahlem Seed Bank, Botanischer Garten und Botanisches Museum Berlin	collected in the wild	Germany: Brandenburg, Sachsen-Anhalt, Sachsen, Thüringen	21	29683
		collected in the garden	Germany: Brandenburg, Baden-Württemberg	12	58342
Germany	KIT Karlsruhe Institute of Technology	collected in the wild	Germany, Baden-Württemberg, Schwäbische Alb	30	unknown
Germany	Loki-Schmidt-Genbank, Botanischen Garten Universität Osnabrück	collected in the wild	Germany: Hessen, Nordrhein-Westfalen	29	unknown
Poland	Kostrzyca Forest Gene Bank		Poland	2	unknown
Poland	Polish Academy of Sciences Botanical Garden Center for Biological Diversity Conservation in Powsin		Poland: Opolskie, Śląskie, Lubuskie	5	3150
Poland	Silesian Botanical Garden		Poland: Opolskie, Śląskie		unknown
United Kingdom	Millenium Seed Bank	collected in the wild	United Kingdom: England: Sommerset	2	6073

Tab. 3: European seedbanks with accessions of *Dianthus gratianopolitanus* according to Ensslin et al 2024.

Ex-situ cultures in Botanic Gardens

Ex situ cultures in gardens are regarded as a useful back-up for *Dianthus gratianopolitanus* (Jańczyk-Węglarska ez al. 2013, Koch et al. 2021, Zippel et al. 2021a). In Poland in the Botanic Garden Poznań takes care for one conservation culture (Jańczyk-Węglarska et al. 2013), in Germany there are cultures in the Botanical Gardens Berlin, Chemnitz, Dresden, Heidelberg, Mainz, Potsdam, Regensburg, Tübingen with known wild provenance. To avoid hybridization between different provenances, most of the gardens hold just one population (VBG 2024).

2. Aims of rescue plans

The aim of species conservation is to develop stable populations of the target species with natural regeneration. Such viable populations produce sufficient seeds to keep the generative regeneration going ("seed rain) and have the genetic diversity that allows the population as well as the species to adapt to changing environmental conditions.

The habitat is therefore the primary focus of species conservation efforts, its conservation and protection as well the expanding, where possible. For populations of *Dianthus gratianopolitanus*, this mostly means use or management adapted to the species, such as grazing and the removal of emerging woody plants. Particular attention must be paid to ensuring that the population can rejuvenate in its habitat. Particularly in extreme locations such as those where *Dianthus gratianopolitanus* grows, attention should be paid to a variety of microclimatic niches in order to offer the species suitable locations in which germination and establishment of young plants can take place in view of capricious weather and climate change.

Many populations of *Dianthus gratianopolitanus* consist of only a handful of individuals. With decreasing seed production ("seed rain"), the probability of generative rejuvenation also decreases. Genetic processes such as genetic drift and inbreeding depression can weaken the fitness of the plants, and the often low genetic variability of the populations may it make difficult or impossible for adapting changing climatic conditions. Here, measures such as population support by sowing or planting of individuals from ex-situ propagation of regional origins may be appropriate. The establishment of new populations can help with biotope networking and establish genetic exchange between populations via pollen and seeds to increase genetic diversity and thus better adaptability in the future.

3. Suggestion of interventions

3.1. Site management

The existence of *Dianthus gratianopolitanus* depends largely on the land use and / or the biotope management of the sunny, nutrient-poor extreme locations. Many populations on rocks with their shallow soils that dry out extremely in the summer months remain stable for decades. In these areas, regular monitoring and, if necessary, occasional de-bushing at intervals of several years will be sufficient.

However, other populations of *Dianthus gratianopolitanus* require an adapted management, especially in view of the high nitrogen emissions and the current climate changes. Other rare, low-competitive, endangered species of rocky meadows that are associated with *Dianthus gratianopolitanus* will also benefit (see chapter 1.7).

3.1.1. Grazing

Where populations of *Dianthus gratianopolitanus* have been able to persist or establish themselves due to centuries of the influence of livestock, grazing can be the optimal way to maintain the habitats.

As a rule, grazing is carried out at a time when the seed maturation of the target species is complete. However, it can be helpful to graze at least occasionally at an earlier stage in order to prevent the formation of closed lawns, to weaken competitors and to create patches of bare ground. Occasional grazing, even during the flowering period, should not weaken a population, allowing it to flower and bear fruit again in subsequent years. Jäger (2013) recommends short-term grazing several times a year using goats or sheep in herding systems.

Too intensive grazing, especially by goats, can have a negative impact on *Dianthus gratianopolitanus* populations (Banzhaf et al. 2009). Particularly in areas where the rocky habitats are located in extensive areas of dry and semi-dry grassland and where long-term grazing is practised, the sites with *Dianthus gratianopolitanus* should be excluded from grazing after a short period to enable the regeneration of grazed cushions and to reduce trampling damage. In exposed rocky locations, the cushions should be fenced out completely if necessary.

3.1.2. Tree cutting

Where grazing is not sufficient or not possible, woody plants can removed manually. The essential thinning of shaded locations must be done carefully. Excessive clear-cutting should be avoided. Selective cutting of trees or single branches is the best forestry practice for the conservation of the vegetation of exposed rocky sites (Müller et al 2006). Fast-growing shrubs (e.g. *Ligustrum vulgare, Rosa*- and *Rubus* species) as well as woody weeds like *Robinia pseudoacacia* trees should be removed with their roots where possible. Regular aftercare and removal of competing species is essential, at least 2-3 times during the vegetation period as required (Banzhaf et al. 2009).

Succession with shrubs and trees quickly contributes to changes in soil conditions, to humus enrichment as well as changes in the microclimate which lead to less exposure to wind and less blow-out of organic material, which in turn promotes the succession. Shading shrubs should therefore be removed at an early stage.

After the removal of woody plants, the accumulation of humus, which is favored over a longer period of shading and foliage entry, can provide favorable starting and development conditions for competitors, especially grasses and perennials, even in places where *Dianthus gratianopolitanus* was very vital before the woody plants appeared. Therefore, even after thinning, control and care is necessary, which includes not only the removal of emerging woody plants but also the removal of competing herbaceous species. Based on experience (Chapter 1.7.1), adapted grazing appears to be optimal. Where this is not possible, the only option is regular manual maintenance, which must be carried out every few months to years, depending on the location.

Particular attention should be paid to the young growth of trees at the foot of rock faces and rock heads, which can grow tall within a few years and shade the growth sites of *Dianthus gratianopolitanus* and destroy them in the medium term. Regular clearing should be carried out in such places.

3.1.3. Burning

There is no experience of the effects of vegetation burning on populations of *Dianthus gratianopolitanus*. Due to the fact that most populations have few individuals, burning in the stand itself is unlikely to be a suitable measure and should be avoided. However, burning vegetation to create new potential growth sites is certainly conceivable.

3.1.4. Fencing

Where populations of *Dianthus gratianopolitanus* are endangered by outdoor activities, skillful management of hikers, tourists and sportspeople and an excellent information policy (see 3.1.5) are required. This not only includes the possible fencing off populations, but also the relocation or shortening of hiking trails and climbing routes.

3.1.5. Education and public relations

As the habitats of *Dianthus gratianopolitanus* are often located in popular outdoor areas due to their scenic beauty and geological features, educating the public about the ecological value of the sites plays an enormous role in their conservation. The focus should not be on individual species, but on the special features and values of the sites as habitats for plants and animals adapted to extreme locations, as well as the conservation challenges of these sites. As many citizens consider massive interventions in habitats to be a destruction of nature and not a nature conservation measure, the purpose of large-scale deforestation should be clearly communicated to the public. Classic information channels are information boards, flyers, and public lectures, which are often produced or carried out in an exemplary manner by relevant NGOs. Involving the public into to the controlling and monitoring of nature conservation measures as part of citizen science projects is a good way for educating and raising awareness for species conservation in general. The fear that the plants could be excavated or seeds collected as a result of disclosure is certainly justified. However, anyone with criminal ambitions in this regard nowadays has easy access to information about the habitats of even the rarest species. It is therefore all the more important to spread knowledge about the occurrence of regional floristic and faunistic treasures among the public, because only what society knows and values will be protected and preserved. Botanical Gardens can play an important und sustainable role in education (Zelenika et al. 2018). With a bundle of offers that range from guided tours, action and course days, specific information on individual rare species, materials for school classes to training for multipliers (e.g. Becker and Hahn 2024), Botanical Gardens can reach a large number of visitors.

In this context, the topic of the introgression of genes from non-autochthonous species or cultivated hybrids must also be mentioned. Although the use of *Dianthus gratianopolitanus* in regional seed mixtures for sowing in the open landscape is prohibited in Germany, seeds and plants of the species of unknown origin are freely available in garden centers as ornamental plants for gardens. Public relations work for *Dianthus gratianopolitanus* must always include the endangerment of the species through possible hybridization with garden plants, the associated loss of genetic diversity of indigenous occurrences and the risk of introgression of foreign genes into isolated relict occurrences.

3.2. Species targeted interventions

Targeted interventions for *Dianthus gratianopolitanus* must be orientated towards its biology and ecology (Käsermann 1999, Banzhaf et al. 2009) as well as its genetics (Putz et al. 2021, Koch et al. 2021) taking into account general genetic principles for botanical species conservation (Borsch & Zippel 2021).

3.2.1. Ex-situ conservation

3.2.1.1. In-vitro culture

In vitro culture has been used for *Dianthus gratianopolitanus* propagation. Fraga et al. (2004) established a tissue culture procedure using shoot tips and nodal segments as explants. After surface disinfection, explants were cultured on Murashige and Skoog (MS) medium (Murashige and Skoog 1962) with 30 g l-1 sucrose and gelled with 7 g l-1 agar-agar. Plant growth regulators (cytokinins and/or auxins) required greatly varied depending on the explant type and genotype. Thus, while in one the genotypes tested, addition of 0.57 µM indole-3-acetic acid (IAA) or 2.46 µM 6-(γ,γ-dimethylallylamino)purine (2-iP) is recommended depending on the explant; in the other one, better results were achieved in the absence of hormonal supplementation. Although high cytokinin concentration promoted shoot production, it increased hyperhydricity and induced phenotypic variation. In vitro rooting was promoted by auxins, although good results were also achieved ex vitro without hormone supplementation.

Cristea et al. (2006) multiplied shoots initiated from nodal and apical explants. The basal medium consisted on the MS formulation supplemented with 20 g l-1 sucrose, 100 mg l-1 myo-inositol, 1 mg l-1 of the vitamins thiamine HCl, pyridoxine HCl and nicotinic acid, and 7 g l-1 agar. The effect of different hormonal combinations (cytoninins and auxins) on the multiplication and rooting processes was tested. Although both medium, supplemented with 1 mg l-1 kinetin and 1 mg l-1 IAA or with 1 mg l-1 kinetin and 1 mg l-1 α-naphtaleneacetic acid (NAA) supported shoots multiplication and rooting, slightly higher multiplication rate was achieved when culture medium contained 1 mg l-1 kinetin and 1 mg l-1 NAA. Contrarily, better rooting was obtained when IAA was the auxin included in the culture medium. In general, shoot segments gave rise to higher multiplication rates than shoot apexes, although rooting was favored when apical explants are used.

3.2.1.2. Seed banks

Because the seeds are orthodox, the storage of seeds is a valuable and inexpensive support for all protective measures for *Dianthus gratianopolitanus*. It is strongly recommended to collect seeds from all populations in situ according to the rules of the European Native Seed Conservation Network (ENSCONET 2009a, 2009b) and store them in local seed banks for wild plants. Most seed banks for wild plants are part of botanical gardens. Following seed banks in the distribution area of *Dianthus gratianopolitanus* are members of the ENSCONET consortium:
Belgium: Plantentuin Meise, Meise.
France: Muséum National d'Histoire Naturelle, Paris.
Germany: Botanischer Garten und Botanisches Museum Berlin, Berlin. Universität Regensburg, Regensburg.

Luxembourg: Musée national d'histoire naturelle, Luxembourg.
Poland: Ogród Botaniczny – Centrum Zachowania Różnorodności Biologicznej w Powsinie, Warszawa. Leśny Bank Genów Kostrzyca, Kostrzyca.
Switzerland: Conservatoire et Jardin botaniques Genève, Genève. Botanischer Garten der Universität Zürich, Zürich.
United Kingdom: Royal Botanical Gardens Kew, London.

3.2.1.3. Plant cultivation

Dianthus gratianopolitanus is very easy to cultivate. Outdoors in sunny locations, the plants quickly grow into strong and healthy cushions. The soil should be a well-drained and alkaline-rich substrate (Cheers 2003), which can also be low in lime (Köhlein & Menzel 1992). In the shade, the species thrives less vigorously, flowers less or not at all as in its natural habitat and the cushions disintegrate into loose mats (own observation).

Plants are obtained by sowing or with cuttings, which root easily during the vegetation period in an unheated greenhouse (own observation). In the Berlin and Potsdam Botanical Gardens, the species has been cultivated for years in natural soil, in sand with a less clay content. Seeds are sown directly in the bed or in seed trays on sand and transplanted into a peat-free mixture of sand and compost with the addition of some clay and shell limestone. Koch et al. (2021) used a mixture of a well-drained substrate consisting of 25 % expanded slate, 25 % pumice, 12.5 % lava, 6.25 % sand, 6.25 % quartz sand, 25 % TKS®1 (Floragard, Oldenburg, Germany), and bentonite clay powder. In view of the ease of cultivation, the use of peat-free substrates is strongly recommended for nature conservation reasons.

To avoid hybridisation with other *Dianthus* species or mixing different origins, it is recommended to hold only one population in the garden. Otherwise, for to obtain seeds for species conservation controlled pollination is necessary. General information on the establishment and care of conservation cultures can be found in Lauterbach et al. (2015).

3.2.2. Plant translocation

Population reinforcement / Reintroduction

Experience to date has shown that the support existing populations with autochtonous material as well the establishing of new populations can contribute to the in situ conservation of *Dianthus gratianopolitanus (*see chapter 1.7.1). Success depends on the choice of planting area, any necessary care and the water supply to the young plants or seedlings.

Assisted colonization and Ecological replacement

The extent to which supported migration and ecological replacement can play a role in the conservation of *Dianthus gratianopolitanus* remains to be discussed, as the fitness of the species is affected by increasing temperatures (Koch et al. 2021), see also Chapter 1.6.2.

Suitable approach for plant translocation

Successful settlements of *Dianthus gratianopolitanus* in Central Europe show that both the expansion of existing populations and the establishment of new populations at suitable locations can be successful and can contribute to its conservation. In view of the highly fragmented occurrences, some of which differ considerably genetically (see 1.2), autochthonous material should always be used to support populations. Accompanying molecular genetic studies are desirable in order to be able to follow the genetic processes of the enlarged or established populations and to draw conclusions for the optimization of future experiments.

Whether seeds or plants are used for reintroductions depends on various species-specific properties (Albrecht & Maschinski 2012) and pragmatic requirements such as available seed quantity, labor, time and equipment (Guerrant & Kaye 2007). Plant release is usually the more successful method with survival rates of around 30% on average (Godefroid et al. 2011; Guerrant & Kaye 2007; Menges 2008).

Careful documentation of the field work is essential. Documenting and marking of planting or sowing sites so that they can be found again months or years later, also by people which were not involved at the beginning of the field-work. Depending on the terrain and soil, stick-on labels (Fig. 12), colour markings or tapes on neighboring trees are suitable for marking. Detailed photographic documentation and the recording of GPS data also make it easier to locate the planting sites.

Sowing

Experience has shown that the establishment rate of seedlings in situ is low to very low in most cases and, according to our own experience and Godefroid et al. (2011), is between 0 and 1%. Therefore, the amount of seeds introduced must be very large. If there are enough seeds available and financial resources are scarce (Godefroid et al. 2011; Guerrant & Kaye 2007, Kaye & Cramer 2003, Menges 2008), direct sowing is possible as well as useful. Direct sowing avoids cultivation and, if necessary, reproduction in the garden and thus artificial selection through gardening (see below). In shallow habitats such as rock heads and rock ledges, placing seeds into specific microhabitats like rock cracks and crevices is the only option.

Seed germination and establishment of young plants is highly dependent on the weather conditions at the time of the experiment. The seeds od *Dianthus gratianopolitanus* are non-dormant, therefore several successive sowings in different weather conditions can be useful.

Transplanting of new individuals

In our experience, large, strong young individuals of max. two years age are best suited for planting out (see also Guerrant et al. 2004, Albrecht & Maschinski 2012). The advantage of spreading rapidly developing young plants over seeds is that flowering specimens will be available the following year and can already serve as seed producers in situ.

For in situ settlements, the cultivation of young plants must be carried out particularly carefully. On the one hand, due to the endangerment of the species, there is often very little and therefore particularly valuable raw material available, and on the other hand, special care is required when gardening. When propagating, there is a risk of horticultural selection, for example through the use of growing substrates and conditions, through selection of young plants according to the time of germination, and the disposal of living but not yet germinated seeds (Ensslin et al. 2011, Lauterbach et al. 2012). Among other things, the choice of substrate, care, appropriate pest control and the avoidance of hybridization must be taken into account (see also Lauterbach et al. 2015) and contamination with pathogens, parasites and neophytes through substrate and cultivation must be avoided.

In order to make it easier for the planted individuals to adapt to the nutrient-poor natural location, it is advisable to approximate the growing substrate to the soil conditions and nutrient ratios of the target area. The substrate in which the plants are grown should correspond to the substrate of the target area in terms of nutrient content and permeability. Young plants grown in small pots are recommended for planting on shallow rocky sites; larger plants can also be planted on sand without any problems.

Fig. 12 Growing seedlings *of Dianthus gratianopolitanus* in a nutrient-poor, sandy substrate

The production of young plants itself is easy. The seeds germinate quickly and easily (see Chapter 1.4.3) and quickly grow into strong plants. If there are not enough seeds available, young plants can easily be grown using cuttings (Zippel et al. 2021a). Growing young plants in multi-pot trays, which are available in various sizes, makes it easier to handle the trays in the garden and transport them around the site.

The number of individuals released depends on the size of the area available and the material available. In general, the more seeds or seedlings planted at appropriately high densities, the greater the likelihood of success (Bontrager et al., 2014; Godefroid et al., 2011; Guerrant & Kaye 2007). Taking appropriate loss rates into account, at least 200 young plants (Lauterbach et al. 2015) should be planted. In practice, however, these numbers are often not feasible and are usually lower. If possible, this can be compensated for by successive settlements over several years. Depending on the success rate of the previous year's measures, the apparently best microhabitats can then be selected for planting as well as sowing.

The water supply of the young plants is of crucial importance for their establishment at the planting site (see above). Therefore, especially where regular watering is not possible in the first year, planting should only take place after abundant rainfall and suitably moistened soil. In view of the increasing dryness in spring, planting in autumn is recommended to allow the young plants to grow before warmer and hotter dry periods. The increasingly shorter days in autumn ensure less evaporation and the morning dew provides additional moisture. Successful trials in Poland show that spring planting can also be successful (Weglarski & Janczyk-Weglarska 2000).

In rock habitats, planting is carried out very specifically in crevices and rock niches that appear suitable for the species with a little humus accumulation, which may be marked with colour spray (Koch et al. 2021). In the sandy pine forests of north-east Germany, planting with regular spacing in rows has proven successful (Zippel et al. 2021, Fig. 15). A string with markings at defined intervals helps, on the one hand, to plant the plants evenly and, on the other hand, to find the planting locations again in subsequent years

The planting bed should be thoroughly watered before planting, which is often a particular challenge in exposed rocky habitats. The same applies to subsequent irrigation in the first year after planting, which should also decisively promote the establishment of the planted individuals, especially in dry years (Zöphel & Pfeiffer 2020).

Fig. 14 Growing in multi-pot trays, which are available in various sizes, has proven its worth

Fig. 15 Planting in rows with even spacing - here 50 cm - makes monitoring easier in subsequent years

4. Monitoring of impact of interventions

4.1. Plant populations – genetic and biodiversity monitoring

All in situ colonisations of wild plants are experiments. A well-planned experimental programme and subsequent regular monitoring helps to evaluate the success of these measures and to determine the best approach for the species and the region in the future. Only if the measures are carefully documented and monitored, evaluated, and published, errors be avoided in the future and strategies and concepts can be improved.

Regular and careful monitoring of the supported or newly established populations must be planned in advance of the measures. Ideally, the settlement is planned as an experiment to study scientific questions, e.g. the influence of various factors on the success of a settlement. The documentation must include sufficient information on the respective reintroduction as well as the origin of the material used. In addition to the responsibilities and contact persons for the respective measure and the geographical information on the measure or donor areas (e.g. geographical coordinates, exposure, slope, area size), information relevant to nature conservation (including biotope type, species spectrum, characteristic location factors, current use/maintenance measures, monitoring).

Fig. 16 Temporary marking of the recovered individuals with sticky labels facilitates counting

The monitoring data must be deposited with the responsible authority together with that of the settlement or stored long-term, securely and accessible.

OUTPUTS FOR PRACTIONIONERS

Dianthus gratianopolitanus grows in nature in rocky, rarely sandy and dry habitats with sufficient light. In southern exposures, shading by woody plants is tolerated. On northern slopes, all-day sunlight is required. As a light-demanding and drought-resistant species which is adapted to nutrient-poor habitats, the populations of *Dianthus gratianopolitanus* are exposed to various risks that are today increasingly contributing to the extinction of this European endemic plant species. The quite drought-resistance species disappears with increasing shading or when leaf litter, humus and nutrients accumulate. Then it will be displaced by other herbaceous species. These and other important causes of endangerment with their consequences and the countermeasures against are summarized in the following table (Table 4).

Cause of endangerment	Consequences	Measures
Shadowing due to high trees and / or dense shrubs	Cushions become increasingly loose, no flower. No generative or low vegetative and generative reproduction.	Remove single trees or branches, excessive clear-cutting should be avoided. Fast-growing shrubs and trees should be completely removed where possible. Regular aftercare and removal of competing species is essential (Chapter 3.1.1, 3.1.3, 3.1.4)
High Competition through herbs and mosses, eutrophication and humus accumulation	Death of the plants due to competition	Adapted grazing (Chapter 31.3) and / or restoration of poor and dry conditions through removal of shady shrubs and trees (Chapter 3.1.1)
Covering with leaf litter	Death of the plants	Removal of deciduous shrubs and trees (Chapter 3.1.1)
Small and isolated populations	High risk of population extinction. Maybe lack of fruitification, maybe inbreeding and gene drift	Population support with indigenous material (Chapter 3.2.3), ex situ propagation if necessary (Chapter 3.2.1). Classical and molecular genetic studies may help to detect inbreeding and / or genetic drift (Chapter 1.2).
Extreme heat in south-facing locations	No rejuvenation due to extremely difficult establishment of seedlings	Careful thinning of south-facing sites. Avoid complete clear cutting of trees and shrubs.
Leisure activities (mainly hiking, climbing)	Destruction of the plants	Skillful guidance of visitors; relocating or closing of paths, trails and rest areas, agreements with Alpine Clubs for climbing areas, fencing out the population (Chapter 3.1.5), regional public relations work with information on the species, e.g. information boards and flyers (Chapter 6)

Cause of endangerment	Consequences	Measures
Forest management practices	Technical discussions with responsible foresters and forestry workers	Discussions with responsible foresters and forestry workers (Chapter 6).
Renovation work on castles and ruins	Destruction of the plants	Discussions with responsible construction planners and workers (Chapter 6).
Overgrazing	Destruction of the plants	Reducing of grazing (Chapter 3.1.3), fencing out the population (Chapter 3.1.5).
Excavating plants by gardeners	Destruction of the plants	Public relations work, information on the protected status of species and the legal consequences of violations of nature conservation law. Implementation of regional citizen projects to raise awareness of the local flora (Chapter 6).
Damage caused by wild animals	Weakening or destruction of the plants	Fencing out the population or single plants (Chapter 3.1.5).
Climate change	Maybe weak rejuvenation due to reduced flowering, maybe establishment of seedlings more difficult	Translocation of plants or seeds to moister and more sheltered locations (Chapter 3.2.3). Storing seeds in seedbanks (Chapter 3.2.1).

Tab. 4: Causes of endangerment, their consequences and the most important countermeasures

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GUIDELINES

for species conservation

Rescue plan for *Dracocephalum austriacum* conservation

Hana Pánková, Alice Haveldová

Dracocephalum austriacum

General guidelines for *Dracocephalum austriacum* conservation

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Photo: Tomáš Dostálek

Introduction

Dracocephalum austriacum is a threatened species throughout its range and is classified as endangered in most countries where it occurs. Historically, it was more widespread, but its populations are becoming smaller, rarer, and more fragmented. Proper management is necessary to prevent the extinction of this species.

Figure 1. *Gentiana lutea* L. (photo: Snežana Dragićević)

SCIENTIFIC PART

1. General information about the species

1.1. Taxonomy

1.1.1. Nomenclature

The species *Dracocephalum austriacum* L. from the Lamiaceae family can be found under several synonyms: *Ruyschiana laciniata* (Mill., Gard. Dict., ed. 8. n. 2 1768); *Zornia partita* (Moench, Suppl. Meth. (Moench) 139, 1802); *Dracontocephalum laciniatum* (Mill. St.-Lag., Étude Fl., ed. 8, 2: 671, 1889); *Ruyschiana austriaca* (L. House, New York State Mus. Bull. Nos. 243-4, 67, 1923). The species *D. austriacum* belongs with its closest relative *D. ruyschiana* to the subgenus Ruyschiana (Bentham 1836).

It can also be found under several vernacular names: Austrian dragonhead (English); Österreicher Drachenkopf (German); Tête de Dragon d'Autriche (French); Melissa austriaca (Italian); včelník rakouský (Czech); včelník rakúsky (Slovak); Máточник австрíйський (Ukranian); osztrák sárkányfű (Hungarian); Mătăciune (Romanian).

1.1.2. Variability

D. austriacum is variable in its range in terms of quantitative characters. The variability of the species reflects not only the different ecological characteristics of the habitats but also their geographical location. Individuals could differ in the size of vegetative and generative organs. For example, the plants growing on bare rocks are overall smaller and older with a smaller number of stems, which are elongated (Lopes 2015), growing in shade is also linked with reduced vitality (Frank et al. 2015). Plants with different crown colours are rarely found. The colors range from various shades of blue, purple-blue to pink or white (Vít 2021, (Dostálek 2009). In the Iberian populations (Sierra del Cadí mountains), the predominant plants have significantly less hairy stems and leaves and less dissected leaves than plants in the rest of the range (Morales 2010). According to the authors of the main European floras, no intraspecific taxa are known.

1.1.3. Karyology

D. austriacum is a diploid species with 2n=14 chromosomes and a nuclear genome size of 2C=1.36 pg (Šmarda P et al. 2019, Hrouda 2000). Genomic GC content is 39.6 % (Šmarda et al. 2019).

1.1.4. Hybridization

The possibility of hybridization of the *D. austriacum* with other species has never been documented in the literature. Although it is an attractive plant from the point of view of gardeners, there are no hybrid cultivars on sale. However, seeds of this species are commonly available for purchase in garden supplies. Theoretically, the natural hybridization of two European species (i.e. *D. austriacum* and *D. ruyschiana*) should be considered as both species naturally grow sympatrically in Switzerland and France (MNHN & OFB 2003-2023a, MNHN & OFB 2003-2023b, Lauber et al. 2018). In addition, both species are closely related (they belong to the same section) and have the same number of chromosomes, which may increase the likelihood of hybridization (Vít 2021). Within the entire genus, hybridization has only been confirmed between species of a different section in the Middle East (Koohdar et al. 2019).

1.2. Genetics and genomics

The genetic variability of populations of *D. austriacum* was studied using isoenzyme analysis by (Dostalek et al. 2010) in the Czech Karst, Moravia and Slovak Karst. Ten enzyme systems were analysed in 22 plants from each population. Overall, the genetic variability of Czech populations is lower compared to the populations in the Slovak Karst, which may be due to a boundary effect - the location of the Czech populations at the northern edge of the range. No correlation was found between population size and the level of genetic diversity. Genetic diversity is also not correlated with seed production.

The greatest proportion of genetic diversity was concentrated in the populations themselves (over 80%). The Haknovec, Kodská stěna and Velká hora populations appear to be the most valuable populations in terms of genetic diversity, but if we would like to preserve the entire genetic variability of the Bohemian Karst, it is necessary to include Radotínské údolí among the priority sites.
No other published studies from other countries are known.

The risk of outbreeding depression must be taken into account when cultivating *D. austriacum* and by possible reintroductions. An important prerequisite for assessing the degree of outbreeding depression is the isolation of populations and their diversity. A study looking at different pollination types in dragonhead colonies found that seed production was highest after plants were pollinated with pollen from another location. This indicates the absence of the phenomenon of outbreeding depression in the Bohemian Karst (Vít 2021).

1.3. Species distribution and conservation status

1.3.1. Species area

D. austriacum is a steppe species with a Sarmatian-Pontic range, with its center of occurrence in southern and eastern Europe. Historically, *D. austriacum* may have been a much more widespread species and may have survived during the ice ages in its present-day range or outside glaciated parts, e.g. the Alps (Vít 2021). The species occurrence in western and central Europe has often prealpine character. It occurs in sparsely scattered, often widely separated or isolated ranges, from eastern Spain (Serra del Moixeró), France (multiple populations in the western and central Alps), Italy (Lombardia, Trentino Alto Adige, Piemonte), Switzerland (Lower Engadin and Lower Valais), Austria (Lower Austria), Czech Republic (Czech Karst), Slovakia (Slovak Karst, Slovak Paradise), Hungary (Aggtelek Karst), Romania (Transylvania), Russia and Ukraine (Ternopil, Lviv, Chmelnyckyj) to the Caucasus (eastern Turkey, Georgia, Dagestan, Armenia).

The species is listed in the Bern Convention (Convention on the Conservation of European Plants, Wild Fauna and Natural Habitats) as a protected plant species (Annex I). The species is listed in Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats, wild fauna and flora as a species requiring special area protection (Annex II).

In the latest version of the European Red List of Vascular Plants (Directorate-General for Environment (European Commission, 2011) *D. austriacum* is listed in the DD (data deficient) category due to lack of information for the species.

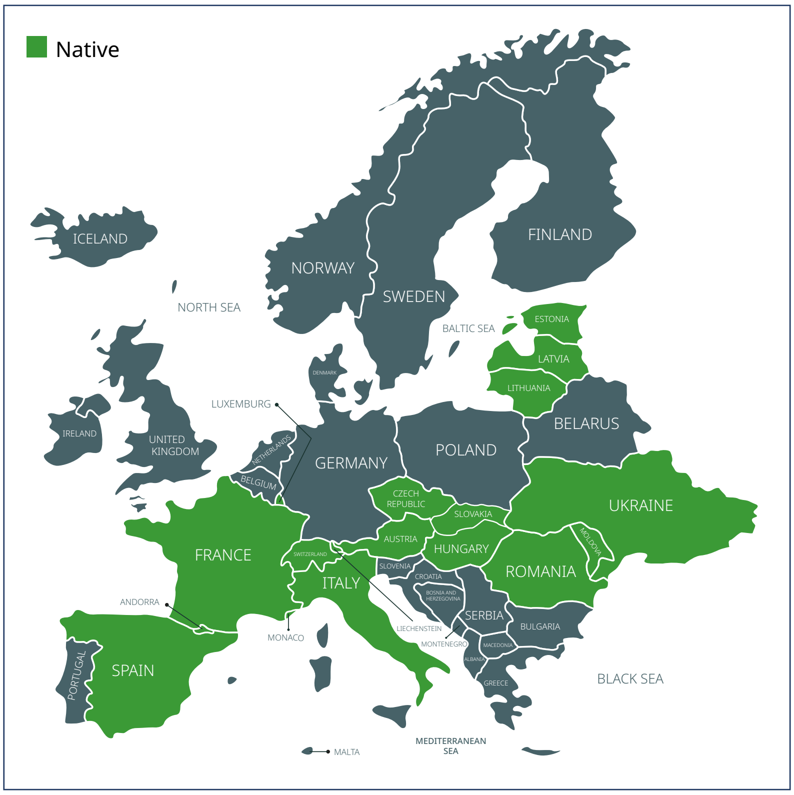

Fig.2 Distribution of D. austriacum in Europe

1.3.2. Occurrence, conservation status and threat in particular countries

1.3.2.1 Czech Republic

The *D. austriacum* is very rare in the warmest areas of Bohemia and in one Moravian locality. The current occurrence is concentrated on the basal substrates of the Bohemian Karst. There are ten known recent localities of the *D. austriacum*. All sites are located in the first zone of the Bohemian Karst Protected Landscape Area and are part of the European sites of European importance (Karlické Valley SCI, Karlštejn - Koda SCI and Radotínské údolí SCI). Roughly half of the populations are very small (a few units to tens of individuals), the other half of the populations are of medium size comprising higher tens to hundreds of individuals (Dostálek & Münzbergová 2011). In the Bohemian Karst, the *D. austriacum* has been reported from other localities that have already disappeared or become extinct. In addition to the Bohemian Karst, the *D. austriacum* was historically reported from three other areas: the Bohemian Central Highlands, the Hustopečská pahorkatina, the Central Povltaví and České středohoří (Vít 2021).

Fig. 3 Population of D. austriacum in Czech Karst

In the legislation of the Czech Republic, the *D. austriacum* is classified as a specially protected species (Decree No. 395/1992 Coll.) in the critically endangered category. In the Red List (Grulich & Chobot 2017), it is listed as EN – Endangered. Recent populations of the *D. austriacum* are found only in the Czech Republic in the Czech Karst Protected Landscape Area. Here, the localities are part of National Nature Reservation and SCI Koda (CZ0214017) (Kodská stěna, Císařská rokle), National Nature Reservation Karlštejn (Haknovec 1 and 2, Kozelská rokle, The rocks opposite Kubrychtova bouda, Velká hora), Nature Reservation and SCI Karlické údolí (CZ0214002), Nature Reservation and SCI Radotínské údolí (CZ0114001).

Bohemian Karst

There are currently only 10 known sites where these populations occur. Dostalek (2005) reports only 9 populations, but the tenth one was reverified by J. Mottl in 2013. All of these populations are found on rocky, alkaline substrates, and the sites often have a very high slope (30 degrees or more) and a very shallow soil horizon (up to 10 cm).

Čeřovský et al. (1999) reported the historical occurrence of *D. austriacum* in the immediate vicinity of Prague. The species was found in several unspecified localities in Prague, including Braník, Chuchle, Prokopské údolí, Doutnáč, and Hlubočepy Parc (Vít 2021). Moucha (1990) indicated that some populations in the Bohemian Karst were destroyed by mining, most likely including the population in Petzold Quarry (Kubát 1986).

1.3.2.2 Slovakia

Dracocephalum austriacum is a very rare species in Slovakia. It is found in the southern part of the country. Nowadays it is mainly present on steeper slopes with a basal substrate in the Slovak Karst, although one population can be found in Slovak Paradise (Šefferová et al. 2015). Unconfirmed data on the occurrence of the species are from the Slovenské rudohorie (around hill Kohút), Malé Karpaty (vineyards around Bratislava) and Tribeč (around Nitra; Bertová & Goliašová 1993). Populations are mostly small (1 - 75 individuals, only 35 % of the sites have population size higher than 50 individuals) and most of them are threatened by overgrowth (KIMS 2013). The size of the populations of *D. austriacum* is now mostly stable or steadily decreasing, but the species is not in immediate danger of extinction there (Šefferová et al. 2015). They grow in collinal range at an altitude between 300 and 800 meters in vegetation *Festucion valesiacae* (Bertová & Goliašová 1993) and *Seslerietum heuflerianae* (Virók & Puska 2006).

The *D. austriacum* is included in the list of protected plants of Slovakia according to Annex V, Decree No. 24/2003 Coll. of the Ministry of Environment of the Slovak Republic. In the Red List of Ferns and Vascular Plants of Slovakia (Eliáš et al. 2015) the species is listed as Endangered (EN). The territorial protection of the *D. austriacum* is ensured in the Slovak Karst National Park: in the Domické škrapy SCI (SKUEV0347), Teplice slopes (SKUEV0284), Plešivské slopes (SKUEV0343), Plešivská planina (SKUEV0353), Horný vrch (SKUEV0356).

Slovak Karst

There are in total 5 areas of occurrence (8 localities, 12 subpopulations), all of them are located within Slovak Karst National Park (Šefferová et al. 2015). Most of them are also part of SCIs in the Natura 2000 network (SKUEV0284 Teplické stráne, SKUEV0347 Domické škrapy, SKUEV0353 Plešivská planina, SKUEV0343 Plešivské stráne, SKUEV0356 Horný vrch). Populations in the Slovak Karst have decreased in size in the last 50 years and some of them have already disappeared (Dostalek et al. 2010).

Fig. 4. Population in Slovak Karst

Slovak Paradise

The only population of Slovak Paradise is situated within the Slovak Paradise National Park and SCI SKUEV0105 Spišskopodhradské travertíny (NPR Drevenik). This location represents the northernmost and most isolated occurrence of D. austriacum in Slovakia (Blascakova et al. 2011).

1.3.2.3 Hungary

In Hungary, 10 areas of occurrence of *Dracocephalum austriacum* were previously recorded, in nine of them the species is now extinct (Virók & Puska 2006). Its formerly known populations are extinct in Rákos, Fót, Csákvár, Dabas, Kunpeszér, Kecskemét-Nyír, Vajta, Földeák, Bélapátfalva (Rakonczay 1989). The remaining populations are located in Aggtelek Karst. The plants are currently found mostly on slopes in associations of *Caricetum humilis pannonicum stipetosum pulcherriae, Cleistogenes-Festucetum sulcatae, Polygalo majori-Brachypodietum pinnati* and on the plateau in *Poa badensis-Caricetum montanae.* It was historically also found in *Astragalo austriacae-Festucetum rupicolae, Festuco-Brometea* and *Quercetum petraea-cerris* (Soó 1968; Szerdahelyi 1991). As of 2019, it appears that there are around 1700 - 1800 plants in total (Agrárminisztérium 2019).

D. austriacum is protected by the Ministry of the Environment Decree No 13/2001 (KöM) on protected and strictly protected species of plants and animals. It is listed as strictly protected in the Red List of vascular plants of Hungary (Barina et al. 2007). The territorial protection is ensured in the Aggteleki National Parc, within the Aggteleki-karszt és peremterületei SCI(HUAN20001).

Aggtelek Karst

The Aggtelek Karst area is home to the last seven known surviving populations of a species, with two additional populations that are uncertain (Virók & Puska 2006). Most of these populations are small, with only 5 to 80 individuals, and some have experienced rapid declines in the past, putting them at risk of extinction. There are only two populations that are medium-sized, with around 500 individuals, and one of them was established secondarily (Virók & Puska 2006). Dragonheads mostly grow on south-facing slopes in a zone between rocky grasslands and a hornbeam-oak forest or a natural steppe forest at lower altitudes (around 500 meters, Szerdahelyi 1991, Virók & Puska 2006). Occasionally, they can be found in secondarily established habitats.

1.3.2.4 Spain

In Spain there is one locality where *Dracocephalum austriacum* can be currently found. The plant grows in the Serra del Moixeró mountain range situated in Eastern Pyrenees (Lopes 2015).

The species is nationally classified as endangered (EN) and is legally protected at Catalan level (Annex 1 - "In Danger of Extinction" of the Catalog of Endangered Vascular Flora of Catalonia, Decree 172/2008). The current locality is also within limits of the Natura 2000 Network (ES0000018 Prepirineu Central català SCI) and Cadí-Moixeró Natural Park.

Serra del Moixeró

The single population of *D. austriacum* can be found at an altitude between 1 400 and 1 500 meters (Aymerich 2006, Vigo et al. 2013, Lopes 2015). The current locality is also within limits of the Natura 2000 Network (ES0000018 Prepirineu Central català SCI) and Cadí-Moixeró Natural Park. One part of the population grows on bare rocks with underdeveloped skeletal soils, while the other can be found on well-developed soils (*Berberidion vulgaris, Xerobromion erectis, Festucion scopariae* and *Stipetalia calamagrostis;* Lopes 2015). A small number of plants are shaded by shrubs, mostly by *Buxus sempervirens*. The number of plants has risen from less than 250 to 416 individuals in the last 20 years, which means the population is stable and slightly increasing (Aymerich 2010).

Historically one more population has been observed on the southern slope of the Serra del Moixeró (towards Penyes Altes; Bou 1979, Soriano 1993). The sighting of a few dozen individuals was reported in 1986 (Aymerich 2010), but the population has not been found since (Aymerich 2006). There is a possibility it could still persist there, because of the very hardly accessible area.

1.3.2.5 France

Dracocephalum austriacum is a relatively rare plant in France: 15 populations are known (sometimes 20 are mentioned; BiodiVanoise 2021), distributed in the Alps in the regions Alpes Maritimes, Alpes-de-Haute-provence, Hautes Alpes, Isere and Savoie (Bensettiti et al. 2002). Several of them are found in the 3 Alpine national parks of Vanoise, Écrins and Mercantour and are part of the Natura 2000 network (FR8201751 Massif de la Muzelle, FR8201783 Massif de la Vanoise, FR9301502 Steppique Durancien et Queyrassin, FR9301503 Rochebrune-Izoard-Vallée de la Cerveyrette, FR9301505 Vallon des Bans-Vallée du Fournel, FR9301511 Dévoluy-Durbon-Charance-Champsaur, FR9301524 Haute Ubaye-Massif du Chambeyron, FR9301530 Cheval Blanc-Montagne de Boules-Barre des Dourbes, FR9301552 Adret de Pra Gaze, FR9301499 Clarée). One population is located within Queyras Regional Natural Park. The species has been mentioned but not seen again in different stations: around Mégève and Chamonix (Haute-Savoie), La Salette (Isère), in Turriers, Seyne, La Condamine and Allos (Alpes-de-Haute -provence; Bensettiti et al. 2002). *D. austriacum* also previously had two French populations in the Pyrenees. The first one on Mountain Coronat (northeast slope), which disappeared after the year 1868 (Lopes 2015) and the second in la Font de Coums, which disappeared by the year 1864 (Trotereau 1990, Lopes 2015). Some of the populations disappeared due to pillaging (allegedly by German botanists, Companyo 1864). The species might be in the future reintroduced near its original sites in Pyrenees as a study on the best reintroduction spots was previously conducted there (Lopes 2015).

The list of populations might not be complete as it grows in very difficult areas to access and occasionally outside of protected areas. The populations are scattered, disjointed with variable numbers of individuals: from 7 to more than 1 000, but they very rarely exceed 100 individuals (Bensettiti et al. 2002). It is found in multiple vegetation communities (*Lavandulo angustifoliae-Genistion cinereae, Festucion variae, Stipo capillatae-Poion carniolicae, Xerobromion erecti, Geranion sanguinei*; Bensettiti et al. 2002) at an altitude from 1250 to 2500 meters (Bensettiti et al. 2002, BiodiVanoise 2021), it has also been mentioned to grow as low as at 500 meters (Trotereau 1990).

The species is nationally classified as near threatened (NT) in the French Red List (Olivier et al. 1995, UICN France, FCBN, AFB & MNHN 2018). Regionally in Provence-Alpes-Côte d'Azur (Noble et al. 2015) and in Rhône-Alpes (Conservatoires botaniques nationaux alpin et du Massif central 2015) it is classified as vulnerable. The species is legally protected at national level in Article 1 de l'arrêté du 20 janvier 1982 fixant la liste des espèces végétales protégées sur l'ensemble du territoire (Article 1 from 20 January 1982 establishing list of plant species protected throughout French territory), which means it is prohibited to destroy, pick and sell any part of the plant throughout French territory. The territorial protection is ensured in the three national parks: Vanoise National Park, Écrins National Park, Mercantour National Park, as well as a part of the Natura 2000 network (FR8201751 Massif de la Muzelle, FR8201783 Massif de la Vanoise, FR9301502 Steppique Durancien et Queyrassin, FR9301503 Rochebrune-Izoard-Vallée de la Cerveyrette, FR9301505 Vallon des Bans-Vallée du Fournel, FR9301511 Dévoluy-Durbon-Charance-Champsaur, FR9301524 Haute Ubaye-Massif du Chambeyron, FR9301530 Cheval Blanc-Montagne de Boules-Barre des Dourbes, FR9301552 Adret de Pra Gaze, FR9301499 Clarée).

1.3.2.6 Italy

In Italy *Dracocephalum austriacum* is rare and currently found close to the north border in the Alps in three regions: Piedmonte, Lombardia and Trentino-Alto Adige (Conti et al. 2005, 2006, Aiello 2015). Most (over 1000) of the Italian individuals are concentrated in Trentino populations (Cornello and Monte Malachin; Aiello 2015). The rest of the populations are small and fragmented mostly with under 100 individuals (Aiello 2015, EEA 2022). Most of the localities (except Lombardia and Cornello) are within limits of the Natura 2000 network (IT1160021 Gruppo del Tenibres, IT2040044 Parco Nazionale dello Stelvio, IT3110042 Prati Aridi Rocciosi di Agumes, IT3110043 Prati Aridi Rocciosi di Sant`Ottilia, IT3120114 Monte Zugna, IT3120116 Monte Malachin, IT1160062 Alte Valli Stura e Maira) and the Trentino and Lombardia localities are simultaneously within the National Park of Stelvies.

The species is nationally classified as endangered (EN) in the Italian Red List (Rossi et al. 2013). It is protected by regional law in Bolzano (Annex B of provincial law n°6 of 12 May 2010; Provincia autonoma di Bolzano 2010), Trento (provincial law n.11 of 23 May 2007; Provincia autonoma di Trento 2007), Piedmonte (annex to the Regional Law 32/82 and article 40 of L.R. 19/2009 "Testo unico sulla tutela delle aree naturali e della biodiversità") and Lombardia (regional law n.10 of 31 March 2008, category C1; Regione Lombardia 2010). The territorial protection is ensured in the Natura 2000 network (IT1160021 Gruppo del Tenibres - Val Stura di Demonte, IT2040044 Parco Nazionale dello

Stelvio, IT3110042 Prati Aridi Rocciosi di Agumes, IT3110043 Prati Aridi Rocciosi di Sant`Ottilia, IT3120114 Monte Zugna, IT3120116 Monte Malachin, IT1160062 Alte Valli Stura e Maira). Trentino localities are simultaneously within the National Park of Stelvies.

1.3.2.7 Switzerland

In Switzerland it is found in two regions: Valais (Lower Valais) and Graubünden (Lower Engandine) in west and east parts of the Alps, respectively (Lauber et al. 2018). The species is widespread from colinne to subalpine in an altitude ranging from 550 to 1900 m (Käsermann 1999). It grows mainly in steppe grasslands of *Astragalo-Brometum*, *Stipo-Poion xerophilae*, *Xerobromion* and *Geranion sanguinei.* There are approximately 20 populations in Lower Engadine and around 8 populations in Lower Valais, mostly consisting of small populations. Although some sites have disappeared in the past, these populations appear to be stable. Recently, new populations have been discovered in the Valais region (Käsermann 1999, InfoFlora 2023).

The *D. austriacum* is listed in Switzerland's national Red List as vulnerable (VU) with medium national priority (3; Bornand et al. 2016). The regional status of the species in biogeographic regions Östliche Zentralalpen (East Central Alps) and Westliche Zentralalpen (West Central Alps) is also vulnerable (Bornand et al. 2019). It is legally protected at national level (Annex 2, List of protected plants, Ordinance on Nature and Heritage Protection, January 16, 1991). The Lower Engandine site (Ardez) is a part of the Swiss Emerald network sites.

1.3.2.8 Austria

The species is found in two localities, both in Lower Austria: Wienerwald and Hundsheimer Berge, one population each (Frank et al. 2015). Both populations are small with less than 200 individuals, the population in the Wienerwald recently showed a slight increase probably as a result of habitat improvement. The population in the Hundsheimer Berge is stable. Historically there have been additional known areas of occurrence of *D. austriacum*: on Hohen Wand mountain and at the Starhemberg ruins, but it has long been extinct in both (Janchen 1966 – 1975, Frank et al. 2015). In Austria it inhabits primarily open rocky steppes and steppe dry grasslands in the hilly and lower mountain ranges (collin-submontane) in the altitude between 300 and 500 meters (mainly *Seslerio-Festucion pallenti*; *Seslerietum budensis* and *Geranion sanguinei;* Kasermann and Moser 1999, Ellmauer 2005, Frank et al. 2015). It also occasionally grows in shady gaps in oak forests, but the plants show reduced vitality (Frank et al. 2015). Both sites are part of the Natura 2000 network (AT1211A00 Wienerwald - Thermenregion, AT1214000 Hundsheimer Berge). Additionally in Wienerwald, Verein Freunde der Pertoldsdorfer Heide association continually takes measures for the long-term preservation of the species. The protection of the habitat in Hundsheimer Berge is part of the LIFE project "Pannonian steppes and dry grasslands" (2004-2008).

The species is protected as highly endangered by the Lower Austrian Nature Conservation Act (NÖ Naturschutzgesetz 2000) and listed in the same category in the Austrian Red List (Nikifeld et al. 1999). In Austria, the species is protected in two EVL Hundsheimer Berge (AT1214000) and Wienerwald - Thermenregion (AT1211A00)

1.3.2.9 Romania

The species is found only in Transylvania in two areas: Trascău Mountains and the Postăvarul Mountains (Sârbu et al. 2007). Suitable habitats on calcareous substrates are rare elsewhere in the country. It grows on the edges of steep slopes or near them, in sunny and windy areas (*Stipo-Festucetalia pallentis)*. The populations are mostly small (15-20), but allegedly stable, although they are not systematically monitored, so some of them might be already extinct (Goriop 2008, Mănoiu & Brînzan 2013).

The species is nationally classified as rare (R) in the Red List (Oltean et al. 1994). It is not a protected species in Romania, but some of its localities are at least declared protected areas. Mount Tâmpa is located in the middle of the Brașov Municipality and has been a nature reserve since 1962, a regime established by Decree no. 949/1962. Currently, the site is part of the European Natura 2000 network (ROSCI0120 Tâmpa Mountain). Cheile Turzii is also a part of the Natura 2000 network (ROSCI0035 Romania Cheile Turzii).

1.3.2.10 Ukraine
In Ukraine the species grow in the east part of the country in three regions: Ternopil, Lviv and Chmelnyckyj region. There are 6 known areas (GBIF Secretariat 2023) and it has apparently disappeared in other localities (Kotov et al. 1960). Populations are small, occupying small areas with slow renewal caused by low seed productivity. It grows mostly in meadow-steppe groups of *Festuco-Brometea* primarily on the south exposed slopes, sometimes in shrubs of the *Rhamno-Prunion*. Populations are located in protected areas: Medobory Nature Reserve, its branch Kremenetsky Mountains, Northern Podillia National Nature Park and Podilskyi Tovtry National Nature Park.

The species is classified in Ukraine's Red List as vulnerable (VU; Red Book of Ukraine 2021). All known populations are also located in protected areas: Medobory Nature Reserve, its branch Kremenetsky Mountains, Northern Podillia National Nature Park and Podilskyi Tovtry National Nature Park.

1.3.2.11 Caucasus
The majority of Caucasus populations are located in Russia, specifically in Dagestan, Chechnya, Karachay-Cherkessia, and Krasnodar Krai. Several populations are also found around Mt. Elbrus, where it is believed to be quite common (unofficial information). The remaining populations can be found in Armenia, Georgia, and Abkhazia (Seregin 2023, Flora of the USSR). However, it is important to note that data from this area is incomplete and may be outdated, as most of the localities were taken from herbarium entries. The species in Russia inhabit limestone stony slopes, steppe, and subalpine meadows, with altitudes reaching up to 2400 meters.

No protection data are available (Vít 2021).

1.3.2.12 Turkey
D. austriacum is found in the Eastern Black Sea, Erzurum-Kars sub-regions in Turkey.

No protection data are available (Vít 2021).

1.4. Biology and ecology

1.4.1 Phenology
The flowering period of *D. austriacum* varies slightly depending on the region. In the Czech Republic, the plant begins to grow in the first half of April, and the flowering period typically lasts from mid-May to June. In recent years, due to earlier springs, *D. austriacum* has been known to flower as early as the beginning of May (Dostálek & Münzbergová, 2011). The flowering period in the Czech Republic is similar to that of Slovakia, Hungary, and Switzerland (Virók & Puska 2006, Šefferová et al. 2015, Lauber et al. 2018). In Austria, it blooms from April to May (Frank et al. 2015), while in France, Russia, and the Caucasus, it blooms from June to July (Kotov et al. 1960, Bensettiti et al. 2002). In Ukraine, it blooms from May to July (Kotov et al. 1960), and in Italy, it blooms from the middle of May to early July (Brusa & Raimondi 2020)

1.4.2. Life form and strategy
The *D. austriacum* is a hemicryptophyte to chamaeophyte, with regenerating buds at the ground surface. It is an herb or semi-shrub. According to long-term observations, the plants live for several decades.

1.4.3. Reproduction

1.4.3.1. Generative
The flowers of *D. austriacum* are arranged in inflorescences, typically in clusters of six.Multiple flowers are open at once, attracting a variety of insects. Nectar is produced at the base of the crown, attracting both pollinators and nectar robbers. After pollination, four seeds could be developed in each flower, but usually only one to two seeds mature (Dostálek & Münzbergová 2011). Seed production per stem is very low in Czech populations (ca. 4–5) compared to populations in the Slovak Karst (ca. 6–12; Dostálek & Münzbergová 2011). Large adult plants produce ca.

5.5× more seeds than small adult plants. In larger populations of D. austriacum, seed production per flowering plant is higher than in smaller populations, which is characteristic of species whose populations have been reduced due to fragmentation or habitat loss. Similarly, larger populations have higher proportions of developed seeds (Dostálek & Münzbergová 2011).

The hard clusters of fruits ripen during June in Czechia (Dostálek & Münzbergová 2011) and from August to early September in France (Bensettiti et al. 2002). The species flowers regularly each year, but the success rate of generative reproduction is relatively low and varies considerably from year to year, probably due to climatic conditions (Dostálek 2009). A prerequisite for successful reproduction is the presence of a large number of plants with a large number of flowers on the site. In normal years about 40-60% of flowering stems mature to the fruit stage, but in unfavorable years (due to browsing by game and frost) only about 5% (Englisch et al. 2016).

The seeds of *D. austriacums* have a hard shell and can persist in the soil for many years and thus form a soil seed bank (Dostálek & Münzbergová 2011). The *D. austriacum* has physiological dormancy, i.e. seed germination is suspended until chemical changes within the seed begin. Dormancy can be interrupted by exposing the seeds to drought with subsequent low temperatures and humidity (Baskin & Baskin 2014). Fresh seeds of *D. austriacum* germinate very well, germination, however, varies considerably by location (Kubíková 1991). Germination trials conducted in Austria (Englisch et Schumacher 2015) showed poor germination (ca. 7% on average), but after intensive scarification germination increased to 20%. In-situ germination trials conducted in France (Nicolè 2005) also showed very poor germination rate with only 5 germinated seeds, but ex-situ germination was much more successful with 100 % germination (Vanden-Eede & Vinciguerra, 2002–2005).

The species germination is divided into two periods in the growing season – the first starts with rising night temperatures and occurs from early April to early June, and the second germination phase starts after a dry summer period with cooler temperatures and wetter nights from September to December or January (Englisch and Schumacher 2015 in Vít 2021).

For small populations with low genetic diversity, a soil seed bank is of great importance. However, as Handlová and Münzbergová (2006) point out, a seed bank may not be sufficient to rebuild a population that has already disappeared. For seedling emergence and the development of young plants of the *Dracocephalum*, an grassland without competition from other species is necessary. In wetter years, larger numbers of seedlings occur on sites, of which only a small percentage persist into the following year.

1.4.3.2. Vegetative

The plants have the ability to reproduce vegetatively and grow into large clumps. Cut stems can produce roots and new individuals. This ability facilitates the cultivation of *D. austriacum* in culture using cuttings to supplement seed sowing (Englisch and Schumacher 2015 in Vít 2021). The cultivation of *Dracocephalum* using cuttings has already been addressed by Moucha (1990), who reported that plants propagated by cuttings have relatively high mortality (up to 50%), shorter life span and are more prone to freezing during winter (Moucha 1990, Englisch et Schumacher 2015 in Vít 2021). Plants grown from a seed flower in the third year (Moucha 1986, 1990), while vegetatively propagated plants flower in the second year.

1.4.1. Plant life cycle

In the first year after germination, a single stem with leaves develops, the plants expand the following year and may flower in the second or third year. Dostálek & Münzbergová (2013) divided the life cycle of the *D. austriacum* into four successive stages (seed, seedling, small adult plant and large adult plant) and studied the transitions between each stage. The seedling is a plant with one non-flowering thin bulb, up to a maximum height of 10 cm. These are plants that germinated in the year they were first observed or in the year before.

Fig. 5. Seedling od Dracocephlaum austriacum

Small mature plants are those with one bulb more than 10 cm tall or with two to five bulbs. Large mature plants have six or more panicles. In times of unfavorable conditions or due to the age of the stems, the transition from large adult plant to small adult plant may occur (Dostálek & Münzbergová 2013). At that time, the entire aboveground part of the plant may get dry and only the ground buds survive.

The most important stages of the plant life cycle are connected with plant reproduction, i.e. seed production and seedling emergence (Dostálek 2005). Decline in these stages can be a major problem, especially for smaller populations. The rest of stages (survival of seeds, small and large adult plants) are stable between populations and years, suggesting that they are not very sensitive to changes in habitat, even though they have the greatest theoretical impact on population growth rates (Dostálek & Münzbergová 2011). In the research conducted between 2003 and 2006, population growth rates were found to be roughly around the value of 1, indicating stable populations. However, even during this short period, there were years when some populations declined (e.g. the population on the Koda Wall) or experienced larger fluctuations in numbers.

Fig. 6. Large adult plant

1.4.4. Ecology requirements

The *D. austriacum* grows on rocky steppes or on the edges of forested areas. In the Czech Karst it is a differential species of the *Seslerio-Festucion* association, a diagnostic species of the *Helianthemo cani-Festucion pallentis* association and a characteristic species of the *Helianthemo cani- Seslerietum calcariae* Klika 1933 and *Helianthemo cani- Caricetum humilis* (Kubíková 1977, Kubát 1986, Čeřovský et al. 1999). The Slovak populations grow on deeper soils and the predominant vegetation unit is the *Festucion valesiaceae* association (Čeřovský et al. 1999, Dostálek 2009), while in Austria the plant also occurs in the *Geranion sanguinei* association (Englisch et al. 2016). It grows in dry herbaceous borders (together with *Dictamnus albus* and *Polygonatum odoratum*) but does not thrive much, rather occurring at the transitions of these borders and xerothermic rock grasslands (Englisch et Schumacher 2015 in Vít 2015). In Spain the plant grows on two types of soil – on bare rocks with underdeveloped skeletal soils and well-developed soils (within the vegetation units: *Berberidion vulgaris, Xerobromion erectis, Festucion scopariae* and *Stipetalia calamagrostis;* Lopes 2015). French populations are found in multiple vegetation communities: *Lavandulo angustifoliae-Genistion cinereae, Festucion variae, Stipo capillatae-Poion carniolicae, Xerobromion erecti* and *Geranion sanguinei* (Bensettiti et al. 2002). In Switzerland *D. austriacum* grows mainly in steppe grasslands of *Astragalo-Brometum*, *Stipo-Poion xerophilae*, *Xerobromion* and *Geranion sanguinei* (Käsermann 1999). In Hungary, it is currently found mostly on slopes in associations of *Caricetum humilis pannonicum stipetosum pulcherriae*, C*leistogenes-Festucetum sulcatae, Polygalo majori-Brachypodietum pinnati* and on the plateau in *Poa badensis-Caricetum montanae* (Soó 1968, Szerdahelyi 1991). It was historically also found in *Astragalo austriacae-Festucetum rupicolae and Festuco-Brometea, Quercetum petraea-cerris*. In Romania it is found only in the edges of steep slopes or near them in association *Stipo-Festucetalia pallentis* (EEA 2022). Ukranian populations grow mostly in meadow-steppe groups of *Festuco-Brometea* primarily on the south exposed slopes, sometimes in shrubs of the *Rhamno-Prunion union* (Kotov et al. 1960).

Using the Ellenberg Indication Values (EIV) obtained from unweighted averages of phytocenological images for species of the Czech flora (Chytrý et al. 2018), the general ecological requirements of the *D. austriacum* can be characterized as follows:

- light: EIV 9 - plant of fully illuminated sites, occurring when at least 50% of diffused light gets to the ground
- temperature: EIV 7 - the species is a heat indicator, occurring in relatively warm lowlands
- moisture: EIV 2 - species is an indicator of drought, usually viable in frequently drying sites and tied to drier soils
- pH reaction: EIV 9 - the species base and calcium indicator, always growing in calcium-rich conditions
- nutrients: EIV 2 - the species occurs in nutrient-poor sites
- salinity: EIV 0 - the plant is salt intolerant, glycophyte

It is important to note that the EIVs vary slightly based on the country where they were computed and also how they were computed. In Hungary, the EIV for nutrients is 1 (extremely poor), otherwise the values match (Horváth et al. 1995).

In Switzerland the general ecological requirements of the species are (the values are on a scale 1–5) as follows (Landolt et al. 2010):

- light: EIV 4 - luminous
- temperature: EIV 2+ - lower subalpine and upper mountain stages
- moisture: EIV 1 – very dry
- pH reaction: EIV 5 – basic (pH 6.5 - 8.5)
- nutrients: EIV 3 – medium-poor to medium-rich in nutrients
- salinity: EIV – not computed
- continentality EIV 5 - continental (very low air humidity, very large temperature variations, cold winters)

The *D. austriacum* is a heliophyte that grows exclusively on unshaded sites, preferring southern, eastern and southwestern orientated slopes with very high gradients (30 degrees or more; Dostálek 2005a). The species grows on unvegetated upper margins of limestone walls and the edges of rock steppes (Čeřovský et al. 1999) with a very low soil horizon (up to 10 cm; Dostálek 2005a), sphagnum loess (extinct population of Zázmoníky in South Moravia), alkaline basalts (Deblík) and travertine (Dreveník, Slovakia; Čeřovský et al. 1999, Hrouda 2000, Hustáková & Koutecký 2018). It is a competitively weak species that does not tolerate overgrowth by other species.

Populations on steep slopes generally have a lower probability of survival and lower population growth rates than those on more gentle slopes (Nicolè et al. 2011). It is particularly sensitive to excessive lack of rainfall - spring drought causes a reduction in the number of flowering individuals, drought throughout the growing season usually results in low seedling emergence and retention. Additionally, the temperature pattern of the year has an effect - survival and population growth rates tend to be lower in years with low spring and high summer temperatures (Nicolè et al. 2011).

It has also been shown that less exposed and shaded sites host less vigorous individuals, however the proportion of juveniles is usually highest. The most vigorous clusters with more than 20 flowering loci tend to be in open, highly sunlit sites (Englisch et Schumacher 2015). Partial shading by shrubs is not a problem for adult established plants, nor is there a reduction in flowering frequency, but shading may prevent the transition from the juvenile to the adult stage (Englisch et Schumacher 2015). Suitable conditions for plants are found, among other places, at the edge of ravines with mature trees (Englisch et Schumacher 2015), which tend to be microclimatically wetter. Similar experiences have been reported in the Slovak Karst, where bee colonies prefer cooler drained rocky edges of plains (Vít 2021).

1.4.5. Biotic interactions

1.4.5.1. Herbivory

There are no specific pests that would target *D. austriacum*. At the Czech site, beetles (e.g. chafer beetles, *Tropinota hirta*) were occasionally observed during monitoring, but they did not cause significant damage to the populations. At some sites, damage to plants by wildlife may occur - documented (Virók & Puska 2006, Aiello et al. 2015).

1.4.5.2. Pollination

The *D. austriacum* is an entomogamous species that is capable of autogamy (Castro et al. 2015). The species is proteranthic, self-compatible, but an absence of insect pollination leads to production of very few seeds. Thus, self-pollination is possible, but significantly less efficient than cross-pollination. It might be caused by a greater distance between anthers and stigma in flowers, in addition to delayed maturity of male and female generative organs (Castro et al. 2015).

The flowers are visited by a wide range of pollinators dominated by bumblebees (Bombus sp.), mason bees (Osmia sp.) and flower bees (*Anthophora sp*.), however, up to 16 different taxa have been observed visiting the flowers of the *D. austriacum* in the Bohemian Karst (Castro et al. 2015). Similarly, to other entomogamous species, pollen limitation was observed in the dragonhead, i.e. a condition where the quality or quantity of pollen is insufficient to produce an optimal amount of seeds (Castro et al. 2015). Thus, due to unfavorable circumstances, seed production is reduced in the whole population. Kubíková (1991) reports that two, or exceptionally three, seeds normally develop in the flowers of the *D. austriacum*. In a recent study (Castro et al. 2015), focusing on experiments with different pollination methods, the average number of seeds developed was found to range from 0.32 (spontaneous autogamy) to 1.60 (assisted allogamy with the addition of pollen to open pollination) per carpel. Artificial pollination of the flowers slightly increased seed production, but under natural conditions this increase in seed number did not translate into an increase in population abundance. Therefore, Castro et al. (2015) consider *D. austriacum* populations to be demographically stable and current seed production to be sufficient for the survival of the species (based on data from the 2008 growing season). Dostálek and Münzbergová (2011) attribute low seed production to the life form of the species (hemicryptophytes to chamaephytes) - where survival in the adult stage is probably more important for the species than investment in high seed production. Castro et al. (2015) link low seed production to inbreeding, which is particularly evident in small populations (of less than 50 individuals), or to a higher frequency of self-pollination due to lack of pollinators.

Fig 7. Pollination of Dracocephalum by bumblebee.

1.4.5.3.Symbiosis

There aren't any known mutualistic relationships for *D. austriacum* apart from pollination.

In related species *Dracocephalum moldavica* it is reported that under water deficit stress, soil microorganisms can increase plant growth and dry matter production through the improvement of the antioxidant enzyme activities. Beneficial bacteria colonizing plant's roots enhance the solubilization and absorption of nutrients, which ensures a sufficient supply of essential nutrients for the synthesis of chlorophyll as well induce systemic resistance against water stress. This can result in improved water-use efficiency and reduced oxidative damage, which can have a positive impact on chlorophyll content (Ghanbarzadeh et al. 2019, Amini et al. 2023).

1.4.5.4. Antagonism

There aren't any known negative relationships except for herbivory (chapter 1.3.6.1) and competition amongst plant species. *D. austriacum* is a weak competitor and therefore gets overgrown by grasses, shrubs and trees (Dostálek 2005, 2010, Lopes 2015).

1.5. Species threats

The *D. austriacum* is typically a relict taxon, its occurrence pushed into few habitats suitable for the species.

1.5.1. Current

Dracocephalum austriacum currently faces a number of threats. Following threats are classified based on IUCN's list of threats.

K02 Biocenotic evolution, succession

Overgrowth

The predominant threat to *Dracocephalum* populations is presumably habitat loss resulting from overgrowth by shrubs, grasses, and trees. The overgrowth of clearings where this species resides poses a threat to the populations located in Cengles, Prato allo Stelvio, Albaredo, Cornello, and Monte Malachin in Italy. This is particularly evident in the Bolzano populations, whereas those in Trento persist due to a presence of a deer shooting line, kept partially clear of expansive vegetation exclusively by local hunters (Aiello et al 2015). The species is also threatened by natural succession, overgrowth of scrub and expansive herbs in all Czech locations (Čeřovský et al. 1999, Machová et Kubát 2004). Several plant species are capable of outcompeting *D. austriacum*. In Slovakian communities these are shrubs *Prunus spinosa, Spirea media* and *Juniperus communis*, herb *Polygonatum odoratum* as well as grasses (Dostálek 2005, Kims 2013). The Swiss population located near Ardez and the Austrian population in Wienerwald are endangered by an herb *Laserpitium siler* (Käsermann 1999), Frank et al. 2015). In addition, the Austrian population is also threatened by needle litter of the *Pinus nigra* (Frank et al. 2015). In Hungary the species faces competition from the grasses of genus *Stipa (S. pennata, S. joannis, S. tirsa*; Vargané 1993, Sipos & Varga 2014), as well as a shrub *Cerasus fruticosa* and herbs *Teucrium chamaedrys* and *Inula ensifolia* (Sipos & Varga 2014). In France, Switzerland, and Spain, the survivability of populations is also at risk due to overgrowth; however, it doesn't appear to be a primary concern (Lopes 2015, InfoFlora 2023).

Fig 8. Overgrown locality of Dracocephalum austriacum

K04 Interspecific floral relations

Herbivory and grazing

Herbivory does not appear to pose a significant risk to the species, as only a small number of populations are primarily threatened by herbivory. Nevertheless, it contributes to a decline in number of individuals. In Hungary, for instance, the population at Nagyoldal site has been consistently decreasing due to various factors, among which is a high number of herbivores (Vargáné 1993, Virók & Puska 2006). Several other populations in Hungary, France, Switzerland, Romania, Ukraine and Spain are believed to experience adverse effects from the grazing and trampling of livestock (Kotov et al. 1960, Bensettiti et al. 2002, Goriup 2008, Lopes 2015, InfoFlora 2023).

Fig. 9. The stems of Dracocephalum austriacum damaged by wild animals.

In the Karlické Valley of the Czech Republic, damage to plants by wild animals (mouflons) has been observed. In contrast, the existence of herbivores can indirectly maintain the habitats of *D. austriacum* as Italian populations in Trento survive thanks to the presence of a deer shooting line, kept partially clear of bushes exclusively by local hunters (Aiello et al. 2015).

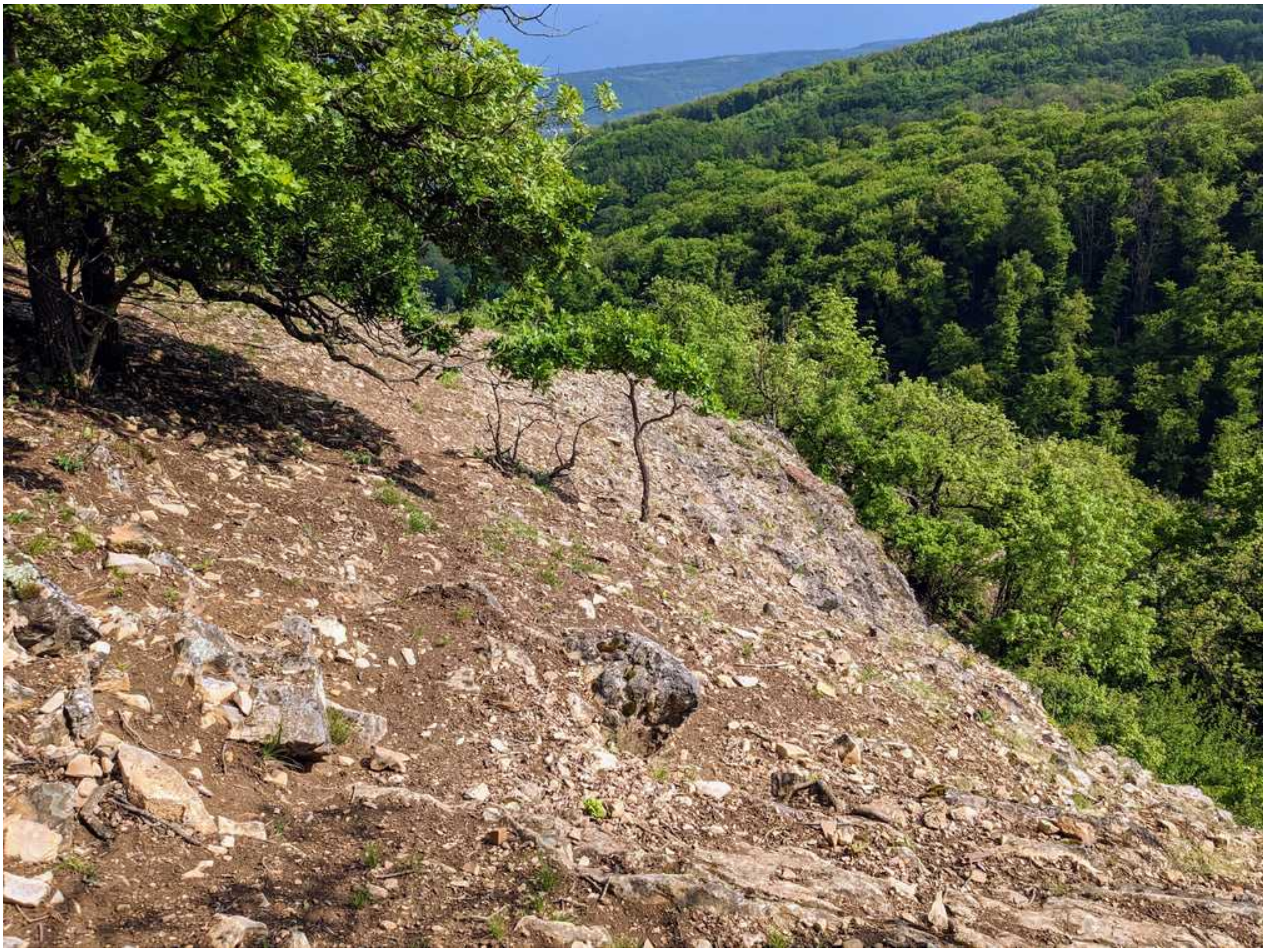

Fig. 10. Locality in Karlické valley destroyed by mouflons.

M01 Changes in abiotic conditions

High temperatures

As average temperatures continue to rise, the potential threats to *D. austriacum* populations must include the negative effects of drought and high temperatures. A study conducted at seven sites in the French Alps revealed that the increase in years with low spring and high summer temperatures resulted in lower survival and population growth rates. In addition, the adverse impact of high summer temperatures varied depending on the quality of the local habitat, with populations on steeper slopes experiencing more severe negative effects. However, higher spring temperatures generally mitigated the negative effects of high summer temperatures (Nicolè et al., 2011).

Drought

Drought might pose a bigger threat than high temperatures. For instance, the Domica population in Slovakia experienced significant negative impacts due to drought in 2022 (KIMS, 2013) and since about 2015, all Czech populations have been significantly affected by drought, which may have contributed to declining plant numbers and lower seed production. The Spanish population faces drought as the main current threat, and it poses at least a minor threat to most other populations (Aymerich 2006). Since 2015, a steep decline in the number of individuals has been observed in the Bohemian Karst at almost all locations, which is most likely related to the extended period of drought (although not exclusively). Particularly dry springs seem to be detrimental to the populations. With regards to the species' habitat (sunny slopes on shallow alkaline soils), it is anticipated that their numbers will continue to decline in the event of continued dry years. Annual droughts most likely do not threaten the long-term survival of populations if they alternate with normal years. In the 1990s in the Czech Republic, a 90% decrease in population numbers occurred due to a one-year lack of rainfall. However, within two years, the population numbers had returned to their original levels (Vít 2021.).

M02 Changes in biotic conditions and K02 Reduced fecundity / genetic depression

Small and fragmented populations

From a population-ecological point of view, small populations of the *D. austriacum* are mainly threatened by demographic and environmental stochasticity. Populations of at least 50 individuals are not considered threatened under proper management but are threatened with extinction if current site conditions do not change (Dostálek & Münzbergová 2011). However, populations of ten or fewer individuals are significantly threatened. In these populations, the probability of extinction is high due to possible random events. The transitions between life stages (seed production and seedling emergence) whose variability contributes most to the actual variability in population growth rate should be the target of management actions (Dostalek et al. 2010). These transitions appear to be most affected by climate change and the expansion of woody species on the sites. At the same time, variability in population growth rate is also negatively affected by reduced genetic diversity and subsequent inbreeding. Due to the vast majority of populations not only being small but having island-like distribution with large distances between individual locations, the cross pollination and in turn gene flow is even more restricted. In France, university research has shown that the low genetic variation of small, isolated populations, such as that of Bessans, can contribute to the decline of the species (Nicolè 2005). A similar scenario is observed in Austria, where the populations are found in two isolated areas, negatively impacting the gene flow between them (Frank et al. 2015). The sole known Spanish population in the Moixeró mountain range is arguably the most severe case of isolation. The closest population was once located in the French region of the Pyrenees, but it became extinct in 1868, resulting in complete isolation for the Spanish population (the nearest populations are now found in the Alps). However, the genetic risk due to isolation and the small size of the population is not considered to be a threat (Aymerich 2010). The populations in Slovakia are predominantly small (65 % sites have populations with less than 50 plants) and while not as isolated, few populations are also impacted by reduced genetic diversity, in relation to low seed production (KIMS 2013, Šefferová et al. 2015).

F04 Taking / Removal of terrestrial plants, general

Collection of plants or seeds has historically been problematic due to species' high attractiveness and horticultural value to gardeners. For instance, the population at Nagyoldal declined significantly, and one possible explanation for this decline was the suspected illegal harvesting of seeds, as reported by Vargáné in 1993. Similar experiences are also reported from other regions within its range, including NP Slovenský kras (Vít 2021), Austria (Ellmauer 2005) and Switzerland (InfoFlora 2023). These areas have suffered or are still suffering from significant damage from uprooting. The populations on Deblik (Vít 2021).) and Zázmoníky in Czechia were probably lost due to plant removal. In France, the plant grows on steep slopes, which creates challenges for picking and uprooting. Despite the difficulty, harvesting still occurs (Bensettiti et al. 2002, Nicolè 2005).

G01 Outdoor sports and leisure activities, recreational activities

Populations situated near recreational areas (such as Wienerwald) or hiking trails are at risk due to increased trampling. For instance, the Piedmonte population in Italy is easily accessible as it is on the periphery of a dirt road, making it vulnerable to damage by vehicles, tourists, and botanists (Aiello et al. 2015). This issue is prevalent in numerous other countries including Spain, France, Hungary, Slovakia and Czechia. Romania considers it a primary threat (Goriup 2008).

E01 Urbanised areas, human habitation & E06 Other urbanisation, industrial and similar activities

As the majority of populations reside within protected areas, the development of new infrastructure poses a minimal threat. In Ukraine and Czechia, some populations vanished due to construction activities, mainly limestone quarries (Kotov et al. 1960). Direct destruction of plant habitats in Czechia may occur in the future during the reconstruction of the railway line between Prague and Beroun. Construction works are planned on the rock walls directly above the line where the species occurs, mainly at the Na Vanovice locality. A potential expansion of a runway presents a low-probability risk for the population in Spain (Lopes 2015). There is also a potential threat of tourist infrastructure expansion in Switzerland (InfoFlora 2023).

L05 Collapse of terrain, landslide

The Lombardia and part of Piedmonte populations are situated on a steep incline, making them highly susceptible to geomorphological instability (Aiello et al., 2015).

1.5.2. Future

Most of the current threats are predicted to persist, with some expected to intensify. Few new threats are anticipated to emerge.

M01 Changes in abiotic conditions

Drought and high temperatures

Average temperatures are likely to continue to rise in the future, which, when combined with drought, may have a more severe impact on *D. austriacum* populations than at present. Given the current effects of drought, seed production is expected to be significantly reduced, while population survival and growth rates are also expected to be low.

Other changes

Unpredictable fluctuations and extreme weather events resulting from climate change can also negatively affect populations by reducing the number of individuals and their ability to reproduce or by altering their habitats. The frequency of landslides may increase due to higher erosion, which could have an impact on populations living on steep slopes.

M02 Changes in biotic conditions

Decline of pollinators

D. austriacum is primarily entomogamous, although it is capable of self-pollination, cross-pollination is much more efficient. Therefore, its sexual reproduction could be negatively affected by a decrease in the number of available pollinators.

F04 Taking / Removal of terrestrial plants, general

As *D. austriacum* becomes increasingly rare, its high attractiveness and horticultural value to gardeners may increase. This could potentially lead to a rise in demand for the plant, which could further threaten remaining populations.

G01 Outdoor sports and leisure activities, recreational activities

Tourism is currently still on the rise, which may put more pressure on populations in accessible areas due to increased tourist visits.

1.6. Previous implemented management interventions

1.6.1 In-situ

Removal of vegetation

Due to its weak competitive ability, *D. austriacum* is often outcompeted by grasses, shrubs, and trees. Therefore, most management regimes require measures to suppress the succession of these plants. At the Austrian sites, woody plants are pruned, *Laserpitium siler* and non-native *Pinus nigra* are removed. These measures have already had a positive impact on the Wienerwald population. Similarly, in Hungary, clearing of woody debris is also carried out. About a decade ago, woody vegetation was removed in the Slovak Karst to support *D. austriacum* populations. However, this intervention, along with subsequent drought, has caused a reduction in the abundance of some populations. Currently, it is preferred to implement small-scale interventions by selectively removing woody debris in specific parts of populations during the year (preferably in autumn or winter) to ensure that trees do not exceed 10% coverage. Similar problem to Slovakia arose in Czechia, where coppicing is the most common measure implemented. However, it appears to be controversial at present. Previously, it was implemented in cycles of several years at a time across the entire site of interest. In the context of prolonged drought, this measure needs to be modified and adapted to the conditions of each site (slope orientation, slope, moisture conditions, etc.).

Fig 11. Locality of Dracocephalum restored by removal of vegetation

Sowing and planting new individuals

In the past, *D. austriacum* have been sown or planted in suitable areas to strengthen populations ore create new . According to Vít (2021), there was an intentional transfer of plants from Velika Góra to Bohemian Karst in the past. One of the current largest populations in Hungary has been artificially created in 1983 using seeds from a nearby site. On the contrary the results of in-situ sowing conducted in 2002 at localities in Hautes-Alpes, Isère, and Savoie indicate a negligible germination rate. Only one germination would have taken place in Lanchâtra (Isère), and four in Bessans (Savoie). The sowing was carried out a few meters from the source population, in a habitat suitable for the species but where it was not currently present (Nicolè 2005). According to Nicolè (2005) and Vanden-Eede and Vinciguerra (2002-2005), it seems that to increase the effectiveness of reinforcement or reintroduction measures, introducing juveniles instead of seeds is preferable. Two protocols with 100% germination rate within fairly short periods of time were established (Vanden-Eede and Vinciguerra 2002-2005). (Godefroid et al. 2010) studied various variables, including the origin of the plant material, the elimination of surrounding plants, and whether the site was protected or unprotected. The results indicate that the survival rate of reintroduced individuals and the success of reintroductions are notably higher in protected sites than in unprotected sites. Some localities were previously enhanced by planting plants directly by the administration of the Czech Karst Protected Landscape Area (Moucha 1990), or by plants cultivated in the Botanical Garden of the capital city Prague (Dostálek 2005). Currently, cultivation and establishment of a backup population to the Velká hora site on Třesina Hill is underway (organized by Třesina z. s.). There might be a new potential reintroduced location in the future in France as a study has been conducted to determine the best sites for the reintroduction of the dragonhead plant in the French part of the Pyrenees (Lopes 2015).

Fig 12. Seedlings of Dracocephalum austriacum established from sowing.

Grazing

Grazing has both positive and negative effects on *D. austriacum* populations. While it can help maintain habitat by removing competing plants, overgrazing by livestock or wildlife can have serious negative effects. For instance, in Switzerland, some localities such as Steinisberg near Ardez were under significant grazing pressure, leading to the implementation of grazing control measures in all areas of occurrence. In contrast, measures implemented in Slovakia include grazing as a means of habitat management. However, according to Šuvada populations thrive even in the absence of grazing and can often be found in the immediate vicinity of hiking trails and can therefore tolerate a slight disturbance.

Mowing

Mowing has an effect on populations similar to that of grazing, but if it is done at the wrong time, it can potentially cause damage. Yearly mowing is a recommended management practice for sites located in Switzerland.

Soil modification

Soil preparation is being carried out at the Austrian sites to improve the germination of *Dracocephalum* by creating suitable habitats. This includes digging up old growth and removing sod.

Fencing

To prevent herbivores from consuming plants, some populations are fenced off. In Hungary, a hedge fence has been built around the Tohonya-bérc population to protect it from horses. The fence had been heavily damaged over time (Virók & Puska, 2006). Similarly, the population in Cengles is also fenced to protect it from deer, but this intervention has been unsuccessful as they can jump the fence (Aiello et al 2015). In Slovakia fencing off sites is a part of the management plan.

Monitoring of populations

Monitoring *D. austriacum* populations is crucial due to the potential for rapid changes in localities and the arrival of new threats. Many countries have their own monitoring plans with varying time intervals. For example, in Hungary, populations are monitored in three-year cycles (Virók et Puska 2006), while in Slovakia, monitoring occurs annually. Austria also includes yearly monitoring of populations in its management program. Monitoring involves counting individuals during visits, typically distinguishing between flowering and sterile (sometimes also fruiting) individuals. Monitoring populations in inaccessible locations, such as Les Têtes and Le Fournel, can be challenging and require alternative methodological approaches. One proposed solution is to establish a permanent transect along one or more booster lines crossing the site (method of vertical monitoring; Bilan 2011).

1.6.2 Ex-situ

Seed banks

Several countries utilize seed banks for ex situ conservation and as a source for species (re)introduction. There are seed banks in France (in possession of CBNA) and Switzerland (Conservatoire et Jardin Botaniques de la Ville de Genève, CJBG; collected from Lower Engandine, Lower Valais).

Backup populations

Some countries choose to maintain backup populations of live plants instead of relying on seeds. Currently, there are backup populations held in several countries. In Austria, the species is cultivated in backup cultivations at Alpengarten Villacher Alpe, Botanischer Garten Universität Wien, and Botanischer Garten Universität Innsbruck. In Czechia, two botanical gardens serve as backup populations for specific sites: the Botanical Garden of Prague (housing plants from Radotínské údolí) and the Botanical Garden and gene pool collections of Průhonice (originally from Císařské rokle and Vanovice). Plants from the Velká hora and Haknovec sites are grown by the Třesina Society and should be planted on the slopes of the Třesina hill in the valley of the Loděnice river. Additionally, a backup population called Zlatý kůň was planted by the staff of the Koněprus Caves Administration in 1992, 2007, and 2008. The CBNA in France additionally to seed conservation collections also cultivates and conducts experimental germination programs for these seeds (Vanden-Eede and Vinciguerra, 2002–2005).

2. Aims of conservation guidelines

The aim of the guidelines is to provide as much information as possible about the species, its threats and possible measures to restore its populations in current area of occurrence. The objectives are based on the fact that the species has recently become extinct in multiple sites across most countries, and it is therefore desirable to prevent any further loss of populations. The plan aims to concentrate on ensuring the care of habitats and the species, while also raising awareness of the species.

The main actions, which should be implemented are follows:
1) To optimise management measures to maximise benefits for the target species.
2) To establish ex-situ cultures of priority populations.
4) To increase the number of individuals on the sites by sowing and replanting.
6) To establish new sites with stable long-term populations.

3. Suggestion of interventions

3.1. Site management

The ideal management of the *D. austriacum* biotope should be to maintain and enhance the steppe character of the biotope. Care should be focused on the continuous removal of woody debris and its deadwood (Dostálek & Münzbergová 2011), considering the higher frequency of dry years. At the same time, attempts should be made to increase the reproductive success of the species, or to increase the likelihood of seed germination and seedling attachment, by creating a suitable environment around the bee clusters. When managing the habitat, it is always necessary to consider each intervention separately and not to be too radical especially in the immediate vicinity of the bees (Englisch et Schumacher 2015 in Vít 2021). In parallel with the care of existing sites, new sites for re/introduction must be identified and prepared. Not only sites identical to the existing ones should be selected, but also sites with slightly different ecological requirements (e.g. slope orientation, soil depth, degree of shading of the site) where the apiary could thrive much better in the context of changing climatic conditions.

3.1.1. Tree cutting

D. austriacum is a light-loving plant that thrives best on sites with a low degree of shading. The removal of woody encroachments therefore appears to be an effective protection of the site against succession. The aim is therefore to strengthen the steppe character of the site. Nevertheless, due to the dry years, there has been a gradual decline in population size on lightened sites. The measure should thus consist of selective removal of a part of the woody debris every (-second) year at the end of the growing season. With caution, application of herbicides to the cutting surface of the caraway or drilling them in is possible. It is considerably more labor-intensive and costly to carry out the measures in longer periods (once every 5-10 years). The result should be a mosaic of steppe with individual solitary trees (e.g. oaks, cranes) providing shade during the day.

Before the intervention, a detailed drawing and briefing of the persons who will carry out the intervention is necessary. At the same time, supervision during the implementation of the measure is recommended, especially if the intervention will be carried out in the immediate vicinity of individuals and used herbicides.

3.1.3. Mowing

D. austriacum is a competitively weak plant species. The dense vegetation thus limits its growth and reproduction. Due to the nature of the sites it is not appropriate to use brush cutting and it is necessary to remove biomass from around the plants by hand. Removing competing plants and accumulated biomass should improve microhabitat conditions for germination and establishment of young plants. A moderate screen of herbaceous plants or low, non-aggressive shrubs does not restrict the apiaries too much.

The measure will involve the removal of encroaching grass growth using a sickle or secateurs and leaf litter by raking with a wooden rake. Low shrubs and clumps of plants (grasses) that may compete with the *Dracocephalum* will preferably be removed. The removed biomass will be removed from the site.

3.1.4. Fencing

In some locations the *D. austriacum* is partly threatened by the movement of visitors or by grazing by wild animals. The fencing should be solid, e.g. from wood to prevent its destruction by animals. The hedge fencing used in other countries was not successful. Nevertheless, because of the rocky background, the fencing of the site is a demanding measure. If such restrictive fencing is close to legal touristic paths, it is recommended to add informative signs justifying the measures used.

3.1.5 Grazing

Grazing as a management measure should be implemented very carefully. It is a useful tool for reduction of biomass on the target site and animals simultaneously disturb the soil, but there is the risk that the individuals of *Dracocephalum* will be damaged. Additionally, the populations of *Dracocephalum* occur on steep slopes, where grazing is very often not feasible. When the grazing will be implemented, we suggest fencing of target plants.

3.2 Species targeted interventions

The care of the species will be mainly focused on the cultivation of the *D. austriacum* in selected botanical gardens. If the rescue cultivations are successful, then it will be possible to reintroduce the *D. austriacum* to new locations, to strengthen the source populations with pre-cultivated plants or targeted sowings. Seeds taken from the cultivations or preferably from the nature can be used for conservation in the genebank. The basis for successful species management is accurate recording of all manipulations of the species, i.e. knowing the origin of all cultivated plants and recording where they have been sown and planted.

3.2.1 Ex-situ conservation

3.2.1.1. In-vitro culture

According to study of (Rasl et al. 2020), in vitro cultivation of *D. austriacum* is possible from seeds as well as from shoots. Cultivation from seeds is based on growth-regulator-free Murashige & Skoog (MS) medium after nicking the seed coat, while propagation via shoot culture on ½ MS medium with 1 µM benzyl adenine (BAP). For successful rooting, medium with 1 µM BAP is suitable. Cryopreservation of shoot tips from cold-acclimated in vitro plantlets and axillary buds from winter shoots from field material is also feasible (Rasl et al. 2020)

3.2.1.2. Seed banks and pollen banks

Storing seeds in national genebanks can significantly extend their usefulness for sowing in case of a sudden collapse of one of the populations of *D. austriacum* in the respective countries. This measure should be carried out in conjunction with cultivation in botanical gardens.

The seeds of the *D.austriacum* can be stored for several years, so their germination period will be substantially extended. Prior to storing the seeds in the designated genebank, it is essential to conduct a germination test on the fresh seeds. This test is a critical parameter for evaluating the success of seed storage in genebanks. The initial germination rate is then compared to all subsequent germination rates during seed storage in the genebank.

The seeds should be dried to 2-4% residual moisture using silica gel and stored in closed doses at -18 °C. The control germination test should be done after two to five years. The optimum amount of stored seeds is 1500 seeds per locality (Vít 2021). In contrary, the seeds in Botanic Garden, University of Agriculture, Vienna are stored on silica gel at -10°C (Kiehn et al. 2009).

There are no pollen banks for the species.

3.2.1.3. Plant cultivation

Ex-situ cultivation of *D.austriacum* is done in botanical gardens. The cultivation could be done from seeds as well as cuttings taken from natural populations. Seed collection is the preferred option, possibly in combination with cuttings. For the establishment of a long-term viable backup population in botanical gardens, it is necessary to grow at least 20 plants of non-clonal origin (i.e. not produced by cuttings from a single plant). The ideal procedure is to grow at least 20 source plants from seeds from 20 different plants from one location. The optimum period for seed is between July and September (Vít 2021). A maximum of 10% of mature seeds (in case of thriving populations) should be collected at the sites according to ENSCONET (2009) recommendations, i.e., annual replenishment of the seed bank at the site will be guaranteed (Englisch et Schumacher 2015). Seed collection must not endanger the source population.

Because of genetic differences, it is crucial to avoid cultivating of plants from different sites in one garden. Additionally, other related species (mainly D*. ruyshiana*) cannot be planted in the garden because of risk of hybridization between species. To keep adaptation to specific environments, creation of adequate soil condition is necessary. The ideal is to grow them on limestone rock or in a humus substrate with a mixture of limestone rubble.

Cuttings can be implemented in sites where few or most of the individuals are non-flowering. The best time for taking cuttings is the second half of June, with the best practice being to break off the lateral shoots (Moucha 1986). Englisch et Schumacher (2015) recommend taking the stems in August, after complete defoliation. Vegetative propagation is successful despite its drawbacks (ca. 50% mortality, poorer wintering in the first years, clonality of cuttings) and is used to strengthen populations, e.g. in Austria (Englisch et Schumacher 2015). It should be remembered that when cutting, clones are used - this can be both an advantage (working with an already selected genotype for the site) and a disadvantage (genetic variability of the population is not changed by cutting; Englisch et Schumacher 2015).

In the Czech Republic the vegetative propagation was tested from the last plant in the Bohemian Central Highlands, but it was not successful (Machová & Kubát 2004). It is thus important to start with vegetative propagation already in vital populations. Collected stems must be put into the substrate for rooting no later than the day after collection. The most suitable soil type is local (humus with limestone crumbles; (Vít 2021).

3.2.2 Plant translocation

3.2.2.1 Population enforcement

In many locations there has been a significant decline in the number of growing *D. austriacum*s in recent years. In addition, the species often grows on very steep slopes, and diaspores are rarely transported to higher elevations. Thus, there is a gradual downward migration of the entire population. Reinforcement by sowing seeds or by plants grown from ex-situ cultures is an appropriate measure to increase the number of individuals on the site and increase the likelihood of its long-term maintenance. For both actions, the origin of population must correspond with the target site.

3.2.2.2 Assisted colonization

D. austriacum is a is tied to the alkaline substrates of the open habitats where population size is decreasing. We can suppose that with predicted climate change these habitats will become unsuitable. The aim of this measure is to find other suitable habitats that might suit more than the existing ones, ideally habitats with slightly different characteristics (e.g. slope and slope orientation, depth of soil horizon, degree of shading). These sites should occur on areas, where the species has previously grown or could grow in the long term. These are primarily drier, loose habitats with deeper soils, less sunny slopes with northern and north- eastern exposure (e.g. abandoned quarries).

The suitability of the selected sites should be assessed by a feasibility study focusing on condition of the source and target sites, the land and the prospects for maintaining of population on the target site in long-term horizon. The feasibility study should be done by sowing approximately 150 seeds in the center of the intended area (approx.

50 cm radius). If the germination and growth will be successful, such site could be considered as suitable for *D. austriacum*. Additionally, the adequate care about the site should be guaranteed. The source population should origin from botanical garden from the nearest site of occurrence (Vít 2021).

The newly established sites should be monitored annually.

3.2.2.3 Suitable approach for plant translocation

Sowing

Sowing is preferable method for plant translocations since natural selection leads to the survival of individuals adapted to the environment. Seeds should be sown during the autumn (late September to early November) in pre-prepared bare soil without sod and stubble. It is advisable to prepare several such sowing plots, ideally 2-4 plots with a radius of about 50 cm(Vít 2021). After sowing the seeds should be lightly incorporate into the soil and water them according to the current weather. Sowing should preferably be carried out at higher elevations on the sites where species has previously grown or could potentially grow. All sowing plots should be documented, and the sites visibly marked on the ground.

Transplanting of new individuals

Planting of pre-grown individuals may be used only exceptionally since it requires regular post-planting care, especially watering and subsequent weeding. Similarly to sowing, plant transplantation should be done preferably in autumn to provide sufficient time for plant rooting. The planting is feasible only on parts with developed soil. The transplanted plants should be marked checked at least three times a year for the first year.

4. Monitoring of impact of interventions

4.1. Plant populations

According to the available information abroad, only the number of individuals and occasionally accompanying vegetation is monitored at the sites. Regular detailed monitoring is carried out only in the Czech Republic. The monitoring is done twice per year according to the methodology (Dostálek 2005)at all sites. At the beginning of May number of flowering and non-flowering individuals is counted. As a one individuum is considered plants that are at least 10 cm apart (Dostálek 2005). In July, number of sterile, flowering and fruiting individuals and number of seedlings are calculated (Vít 2021). Additionally, possible damages of plants, e.g. herbivory, trampling, diseases are evaluated.

The same approach will be applied on new populations or ex-situ cultures.

4.2 Site quality

Evaluation of site quality will focus mainly on the most important threats. The cover and composition of surrounding vegetation will be evaluated on permanent plots.

5. Further research

The main proposed study should focus on the effectiveness of assisted migration and ex-situ cultures. It should combine genetic aspects as well as population dynamic of species. Further, the improvement of conservation measures in context of climate change should be studied. Specifically, following topics should be considered:

- comparison of plant fitness of new plants (transplants or from sowing) and natural individuals
- risks of inbreeding/outbreeding depression, local adaptation
- genetic differenced between ex-situ and in-situ populations
- pollen limitation
- support of pollinators

6. Public awareness

The main aim for this activity is to introduce the species to general public and thus motivate them to its protection. The species could be easily introduced by 3 ways:

- botanical gardens – generally, information about species and its protection are presented near the cultivation. Some botanical gardens create specific thematic family days or create educative programs for children. All these actions could be used for enhancement of public awareness.
- Natural sites – some populations are close to the touristic roads. Thus offering itself to place information table close to the site. At the same time, it must be ensured that the individuals and habitats are not damaged.
- Demonstrative un-natural population e.g. in visitors center or in the nature. Such population could be used for practical demonstration of species without the risk of damage of natural sites. Additionally, it could be more accessible for tourists (Vít 2021).

OUTPUTS FOR PRACTIONIONERS

Q1. Are shrubs and/or trees present in the population?
Yes: Go to Q2
No: Go to Q3

Q2. Is population located on rocky steep slope with south or south-east exposition?
Yes: Remove only the most overgrowing shrubs, keep part of locality shaded
No: Remove all shrubs

Q3. Are there competitive strong species (e.g. grasses, *Polygonatum odoratum, Laserpitium siler)* present in high abundance?
Yes: Go to Q4
No: Go to Q5

Q4. Are the sites well accessible for people and animals?
Yes: Suppress the vegetation by grazing or mowing. Be careful not to destroy Dracocephalum individuals. Consider their fencing
No: Remove the surrounding vegetation manually.

Q5. Are plants damaged by herbivory, trampling or are they collected?
Yes: Fence the population and place close to the fence informative table.
No: Go to Q6.

Q6. Does seedling recruitment occur on the sites?
Yes: Go to Q9
No: Check the habitat quality. If the habitat will be suitable, go to Q7-9, if not, go to Q1

Q7: Are plants producing seeds?
Yes: Go to Q8.
No: Go to Q9.

Q8. Is the soil surface without vegetation?
Yes: Go to Q9.
No: Create small bare soil patches by digging up old growth and removing sod to support seed germination.

Q9. Are populations small (up to dozens of individuals)?
Yes: Consider possible genetic aspect and population strengthening
No: Go to Q10.

Q10. Does the plant population suffer from drought and high temperatures?
Yes: Consider assisted migration.
No: Go to Q11.

Q11. Are pollinators present at the locality?
Yes: The population should be maintained by ongoing management actions.
No: Consider manual pollination.

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APPENDIX

Czech sites

Bohemian Karst

Císařská rokle - approx. 1 km south of the village Srbsko in the municipality of Korno, in the lower part of Císařská rokle on the left slope at the foot of the gorge's marginal wall. N49.929529, E14.131107, approx. 275 m above sea level.

Haknovec 1 and 2- on the NE outskirts of the village of Karlštejn in the municipality of Budňany, on the rocky outcrops of the SW slopes of Mount Haknová. N49.939095, E14.190185; approx. 300 m above sea level and N49.939524, E14.192839 approx. 320 m above sea level. The sites are approximately 200 m apart and are separated by a belt of forest cover.

Kodská stěna - edge of the Kodská wall about 1km W from the village Srbsko,. The site consists of three micropopulations along the south-facing rock wall about 50 m away, groups below the wall + 2 micropopulations about 230 m on the edge towards the west. N49.933892, E14.122956, approx. 350 m. above sea level.

Radotínské údolí - 900 m south of the village of Zadní Kopanina. A rocky ridge of NW orientation on the right bank of the Radotínský brook. N49.998246, E14.311992, approx. 280 m above sea level.

Velká Hora - 1.9 km NE from the village Srbsko. Rocky edges and ridges on the south and southeast slopes of Velká Hora above Kubrychtova bouda. The site consists of 2 micropopulations about 200 m away - the 1st is directly above Kubrychtova bouda and the 2nd is about 200 m to the NW. N49.94707, E14.157018, approx. 300 above sea level.

Karlické údolí - approx. 2 km S from the village of Karlík. It is the upper part of the rock spur on the left bank of the Karlický brook. According to the Discovery database of nature conservation this population was strengthened about 30 years ago, probably by plants from the Kodská stěna. N49.949058, E14.247122, approx. 320 m above sea level.

Kozelská rokle - approx. 1 km south of Hostim. Top of the rock wall on the right bank of the Loděnice River. N49.952587, E14.129348, 235 m above sea level. The population has been strengthened in the past; the current plants come only from these plantings (Vít 2021).

Na Vanovicích - approx. 1 km NW of Krupná. A rocky promontory on the right bank of the Berounka River above the railway line. There are several native plants directly in the wall above the railway line, all others come from plantations (Dostálek & Münzbergová 2011). N49.929483, E14.149725, approx. 230 m above sea level.
The rock opposite Kubrychtova bouda - approx. 1.75 km NE of the village of Srbsko. South-oriented rocky slope 100 m SW of Kubrychtova bouda. N49.946112, E14.155848, approx. 290 m.n. m.

Na Vinicích - approx. 750 m southeast of the village of Srbsko, in k.ú. Srbsko u Karlštejna. South-facing rocky slope. The locality reported in the inventory of Karlštejn NPR (Hummel et al. 2013) numbered 6 individuals., Not verified. N49.931157032, E14.140015929.

Prostřední hora - approx. 1 km W from the village of Karlštejn, in k.ú. Budňany. South-west facing rocky slope. The locality reported in the inventory of Karlštejn NPR (Hummel et al. 2013) numbered 2 individuals. N49.93552594, E14.16821409. Not verified.

Třesina - approx. 500 m south of the village of Svatý Jan pod Skalou, in the municipality of. Svatý Jan pod Skalou. S to SW rocky slopes. N49.9645375, E14.1337456, Not verified.

Hustopečská pahorkatina

In Zázmoníky Nature reservation *D. austriacum* occurred only in one place on the main ridge in the center of the area under the pine trees. In 2011 three plants were observed, in 2018 one sterile individual was growing there, in the following years it could not be found (Vít 2021). Historically 15-20 individuals have been reported here. The disappearance of the species was most likely due to the unsatisfactory condition of the habitat (excessive shading, change in soil pH caused by acidic fallout, lack of management) and, as a consequence, the absence of generative reproduction (Hustáková & Koutecký 2018).

Střední Povltaví

Only two historical sites *D. austriacum* were found in this area: Zvolská Homola and Zbraslav (Vít 2021).

České středohoří

Deblík - On the Deblík hill *D. austriacum* grows at the contact of steppe meadow and oak forest. In 1929, Mittelbach reported about 60 plants in the clearing. However, in 1964 only three clusters were found and in 1984 only two clusters were growing (Vít 2021). In 1995, the last flowering plant remained at the site (Čeřovský et al. 1999), but it has not been found since 1996 (Dostálek & Münzbergová 2011). The site was overgrown with dense shrubs (*Crataegus, Cotoneaster, Rosa*). It is traditionally believed that the site was not destroyed by stone quarrying, as reported by e.g. Moucha (1990) or Sádlo (2002), but the last plant was most likely uprooted by gardeners (Vít 2021). Attempts to vegetatively propagate the last plant before its disappearance were unsuccessful (Machová & Kubát 2004).

Previous implemented management in Czechia

1) Císařská rokle – The site tends to overgrow with woody plants (*Swida sanguinea, Crataegus sp., Ligustrum vulgare, Fraxinus excelsior, Rosa sp., Acer campestre, Juniperus communis, Pyrus pyraster, Carpinus betulus, Cornus mas, Cotoneaster integerrimus)*. In 2014 and 2015, management measures were carried out in the form of cutting woody weeds.

2) Haknovec 1 – The site without care is overgrown with deciduous trees - *Cerasus fruticosa, Fraxinus excelsior, Berberis vulgaris, Rosa sp., Cotoneaster orientalis*. In 2013 the infestations were cut down.

3) Haknovec 2 – The site is overgrown with *Fraxinus excelsior, Berberis vulgaris, Rosa sp., Cotoneaster orientalis, Tilia sp*., in 2010 the scrub was pruned.

4) Kodská stěna – The site is threatened by overgrowth of bushes and trees (*Rosa sp., Quercus pubescens*), especially under the rock wall. The infestations were cleared in 2015 and the site is much more lightened.

5) Radotínské údolí – The site tends to overgrow with woody plants and other species (*Cotoneaster integerrimus, Pinus sylvestris, Sorbus collina, Quercus robur, Berberis vulgaris, Corylus avellana, Juniperus communis, Dictamnus albus*). In 2010, the tree infestations were cleared.

6) Velká Hora – Most of the plants on the site are found in the lower part on a spur overgrown with trees, which may act as protection against excessive drying of the site. They are periodically cut down by encroachments (mostly *Cotoneaster integerrimus*).

7) Karlické valley – Skalka is overgrown with C*ornus sanguinea, Crataegus sp., Ligustrum vulgare, Fraxinus excelsior, Rosa sp., Acer campestre, Juniperus communis, Pyrus pyraster, Carpinus betulus, Cornus mas, Cotoneaster integerrimus.* The infestations are repeatedly cut.

8) Kozelská rokle – The site is overgrown with tree species such as *Cornus sanguinea, Pyrus pyraster, Rosa sp., Fraxinus excelsior, Acer campestre, Crataegus sp., Prunus spinosa, Sorbus collina.* Deciduous trees were partially cut in 2018.

9) Na Vanovicích – The site is overgrown from the east with C*ornus sanguinea, Cotoneaster integerrimus, Corylus avellana, Carpinus betulus, Rosa sp., Berberis vulgaris.* The infestations are regularly pruned.

10) Skalka opposite Kubrychtova bouda – The site is gradually becoming overgrown with bushes and trees from below. In the year 2015, the site was lightened by removing trees

Slovak sites

Slovak Karst

Gemerské Teplice, Štítnik, Plešivecká planina– south and north part, Domica, NPR Zádielska tiesňava

The Domica population grows on a SW-oriented meadow with limestone rocks in NPR and SCI Domické škrapy. It is the biggest population in Slovakia, whose numbers are showing a positive trend (approximately 180 plants in 2005, 200 in 2010, 262 in 2015; Dostálek 2005, Dostálek et al. 2010, Šefferová et al. 2015). It is overgrown by shrubs (mostly *Prunus spinosa*) and grasses to some extent (Dostálek 2005). More recently (2022) the population has been affected by drought, which could have a negative future effect on plant numbers (KIMS 2013). There are at least small-scale interventions on several surfaces, which suppress the succession of shrubs and trees (Šefferová et al. 2015).

There are multiple populations of *D. austriacum* in NPR Zádielska tiesňava (Zádielsky kameň, Okrúhlý laz, Zádielska dolina). Zádielsky kameň is the biggest population (86 in 2005, 100 in 2010, 65 individuals in 2023; Dostálek 2005, Dostálek et al. 2010, KIMS 2013) in this NPR. It is situated on a steep sunny slope on a highest rock in Zádielská planina. The population and habitat status are monitored annually. There are no major negative impacts except overgrowth of shrubs such as *Spirea media* and herbs such as *Polygonatum odoratum* (Dostálek 2005). Otherwise, there is a single medium-sized population (consisting of 36 individuals as of 2023) located on a steep, sunlit slope, alongside two smaller populations comprised of 9 and 16 plants.

Plešivecká planina is a separate karst massif with steep slopes. It has multiple populations. The condition of the species here is either unsatisfactory or bad (Šefferová et al. 2015). Železná vrata is the biggest population on Plešivská planina (110 plants in 2005, 150 in 2010; Dostálek 2005, Dostálek 2010). This locality is on the sunny west rocky edge of Plešivská planina. *Juniperus communis* and other shrubs and grasses gradually overgrow this locality.
In the past, the occurrence of species has been reported in the Slovak Karst also above Drienovec, but recently it has not been confirmed (KIMS 2013).

Hungarian sites

Aggtelek Karst

The oldest known occurrence of *Dracocephalum austriacum* in Aggtelek Karst is from mountain Nagyoldal (above Jósvafő; Virók et al. 2014). The population is located in a zone between a rocky grassland and a hornbeam-oak forest at an altitude of about 550 meters on a SE slope (Szerdahelyi 1991). The number of plants rapidly declined between the early 90s (around 80 plants) and 1996 (a couple dozen plants), further decline followed in the early 00s, which resulted in only 3 plants in 2006 with no new seedling observed (Virók & Puska 2006). Main proposed reasons for the decline are high number of herbivores, suspected illegal seed harvesting, drought and overgrowth of shrubs and *Stipa pennata* (Vargáné 1993). In the past it has been used as a source site for propagating *D. austriacum* to other suitable sites. No measures to save the population were recommended as the species has stable populations elsewhere (Virók & Puska 2006).

The largest secondarily established population (in 1983) from an unknown number of seeds from Nagyoldal locality is located in Tohonya-bérc (Virók et al. 2014) on a SW slope close to the ridge with primarily semi-dry grassland, down the slope becoming heavily shrubby (Virók & Puska 2006). Surrounding it are secondary grasslands and semi-arid shrubby grasslands. By 2006 it has been reported that it has a really strong, expanding population (454 plants) with lots of seedlings. The population is not threatened by natural processes, but the NP’s Hucul horse herd is being grazed in the area, which might have some negative impact through trampling and release of nutrients in horse droppings.

Very close to Jósvafő two other populations can be found: Kopasz-tető and Szőlő-hegy. Other populations are Verő-tető, Szin, Szőlősardó and on the hill Zabanyik. One population used to be split into two, western and eastern, but since 2005 no plants have been found on the eastern side (possibly an effect of shrub cleaning; Virók & Puska 2006). The current population is 82 plants. The habitat is one of the most valuable natural rocky grassland, slope steppe and forest-steppe complexes in the national park. Until the second half of the 20th century the area was cultivated, but original vegetation was preserved in orchards. A probably extinct small population of only 2 plants was found in 1995, but since then despite regular searches no more plants have been found (Virók & Puska 2006). It was located in a managed forest with planted oak trees. They are poorly developed, because of herbivory, edaphic and climatic conditions, which makes suitable open habitats for *D. austriacum.* Another population is growing among poorly growing oak trees. 17 plants were discovered in 1995, the population has grown to 60 by 2006. The habitat between the trees is characterized by a mosaic of rocky grassland and steppe, with many steppe species. In 2002 three populations were found (Virók & Puska 2006). Two of them are very small. The first one had 19 plants by 2006. It is located west of abandoned orchards and vineyards of the Csemer valley, which have been converted into species-rich steppe. The habitat is characterized by forest-steppe vegetation. The other small population (23 individuals) is located on the edge of a planted oak forest. The area is not protected. In contrast the third population is the largest in Aggletek Karst with 457 plants. The habitat is in a natural steppe forest on a slope of a Zabanyik hill with a high species richness. The *D. austriacum* is found at the top of the hill, the area under it was cultivated in the past. The area is not protected as it is privately owned.

Spanish sites

Serra del Moixeró

The single population of *D. austriacum* can be found on a north slope of a Penyes Altes de Moixeró mountain close to the Torrent del Saüc stream in the Moixeró mountain range at an altitude between 1 400 and 1 500 meters (Aymerich 2006, Vigo et al. 2013, Lopes 2015). The current locality is also within limits of the Natura 2000 Network (ES0000018 Prepirineu Central català SCI) and Cadí-Moixeró Natural Park. One part of the population grows on bare rocks with underdeveloped skeletal soils, while the other can be found on well-developed soils (*Berberidion vulgaris, Xerobromion erectis, Festucion scopariae* and *Stipetalia calamagrostis;* Lopes 2015). A small number of plants are shaded by shrubs, mostly by *Buxus sempervirens*. The plants growing on bare rocks are overall smaller and older with a smaller number of stems, which are elongated. The number of plants has risen from less than 250 to 416 individuals in the last 20 years, which means the population is stable and slightly increasing (Aymerich 2010).

French sites

Vanoise

There are 3 areas of *D. austriacum* populations in Vanoise NP (BiodiVanoise 2021). Near the village of Bessans (slopes around river Ruisseau des Houches and river l'Arc), near the village Pralognan-la-Vanoise (two populations: east slope of Mountain Bochor and mountain Le Moriond), and near the village Val-Cenis (two populations: northern one near village Lanslevillard, slope of Rochers de Tufs and southern on slopes of Mont Cenis).

Écrins

NP Écrins has 5 different areas with populations of *D. austriacum* (Biodiv'Écrins 2016 - 2020). South of village Champcella (south slope above Torrent de Tramouillon), west from the village Freissinières (on a slope east from stream Torrent de Naval), near the village L'Argentière-la-Bessée (3 populations: bottom of valley of stream Le Fournel before the break in the slope leading to the Grand Cabane mountain pasture, southeast slope of Les Têtes with limestone slabs, ridge Crête de la Baume noire), northwest from Saint-Christophe-en-Oisans (east slope of mountain La Coche) and Valjouffrey (2 populations near each other: west from town Valsenestre, south slope Tête des Vires). Some populations are rarely visited due to their inaccessible location like Les Têtes and Le Fournel. Les Têtes population is estimated to have between 1 000 and 10 000 individuals, probably thanks to its inaccessibility (Bilan 2011).

Mercantour
There is only one locality in the Mercantour NP (BiodivMercantour 2023). The locality called Saint-Dalmas-le-Selvage is located in the peripheral zone of the national park around and on the La Croix de Carlé mountain, west of the Saint-Dalmas-le-Selvage village. Located at an altitude of around 1,750 m and facing south-east, the site is framed on one side by several hundred meters of limestone rocks, and on the other side by a hay meadow (Lopes 2015). The plant does not grow in full rocky areas, but rather in a hay meadow, on the edge of rocks. It is one of the bigger populations.

Queyras Regional Natural Park

The only population is located in Escoyères (Arvieux). It is not threatened due to its location in a steep and difficult-to-access area. No protection or management measures have been implemented at this station due to the almost non-existent threats. There may be other unknown populations within this park.

Italian sites

Piedmonte
In Piedmonte, there seems to be only one population (10 - 33 individuals; EEA 2022) in the valley of Stura di Demonte, although one other population has been historically reported near the village Moncenisio, but it is possible that the specimens are actually from France (Aiello 2015).

Lombardia
Only one population of the species has recently been reported in the northern part of the Stelvio National Park towards the border with Switzerland on the slopes near the village Livigno (Brusa & Raimondi 2020). The population is made up of two clusters a few tens of meters apart (one above and the other below a wall). In total about 100 individuals. There is one potential area, where dragonhead could occur (upper Valtellina).

Trentino
More populations can be found in the region of Trentino. Three in the province Trento: on slopes of Monte Malachin and Cornello, which are close to each other and a more isolated one near Albaredo (near the village Rovereto; Aiello 2015). The steppe vegetation with *D. austriacum* is present near the rock edges of Monte Malachin facing south (EEA 2022). The Albaredo population is located on the western side of Mount Zugna, on the outcrop of limestone, only partly colonized by vegetation (EEA 2022). Two more populations are located in the province Bolzano: on the slopes near village Prato allo Stelvio (10–30 plants) and the other near village Cengles (30–80 plants; Aiello 2015, EEA 2022). The surrounding area of the population in Prato allo Stelvio is primarily a cultural landscape with meadows and hedgerows. The site is characterized by rocks with pioneer, steppe vegetation and is partly bushy (EEA 2022). The site in Cengles is very difficult to access and was thought to be extinct during the 20th century until it was rediscovered (Aiello 2015). The habitat is characterized by rocks with small arid meadows. The area is surrounded by a mixed forest of *Pinus silvestris*, *Larix decidua* and *Picea abies* (EEA 2022).

Valle d'Aosta
Historically the species also occurred in the Valle d'Aosta, where it was found and collected in village Ollomont in 1834 (Bovio et al. 2014). Since then, the species has not been found, although there are suitable environments.

Swiss sites

Lower Engandine
In Engandine the species can be currently found in one area: Ardez, where around 20 small populations exist (around Muot dal l'Hom, Steinsberg; Käsermann 1999). The Ardez area is protected as part of the Swiss Emerald network sites. *D. austriacum* can be found on warm slopes with herb-rich, slightly bushy semi-dry and dry grasslands with lots of *Laserpitium siler*. In the past, there were two sightings in other localities in Graubünden: Val del Botsh (in 1934)

and Ramosh (in 1995), but no sightings have been reported since. According to unverified information, a small population should also be at the tree line above village Ramosch.

Lower Valais

In Valais, it can be found only in one area: Riddes with around 8 populations (Käsermann 1999). It grows in north-facing, weed-poor, steep dry grasslands from the bottom of valley near Riddes (the only north-facing point) to the tree line in about eight places (boulder above Codo near Mayens de Conthey, in the area of the summit of the Sex Riond, in the rocky bay east of the Sex Riond, between Grand Dzeu and Le Coeur in the Lizerne valley, north of Godey in the Derborence, in a large area near Vertsan Dessous ob Ardon and near Bieudron). Two additional already extinct localities include Dent de Morcles (1940) with one sighting and Nant (1844–1956) with multiple sightings, but no current ones (InfoFlora 2023). Other isolated occurrences are possible in more inaccessible areas in the valleys of La Lizerne and La Morge and possibly near the village Isérables (Käsermann 1999).

Austrian sites

Wienerwald

Wienerwald is mostly dominated by deciduous beech and oak forests, but on the eastern border there are dry grassland areas mixed with vineyards, where the species occurs. Reportedly there are 64–74 individuals (EEA 2022). The species is threatened by *Laserpitium siler* and the needle litter of the *Pinus nigra* (Frank et al. 2015).

Hundsheimer Berge

Hundsheimer Berge hosts a larger population (180–200 individuals) of *D. austriacum* (EEA 2022). It has secondary dry grassland and extensive mesophilic meadows with occasional dense shrubs as well as primary steppe dry grasslands on exposed rocks and steep parts of the hills (with dominance of (*Dictamnus albus, Geranium sanguineum*). The site is on the edge of a forest with *Quercus pubescens*.

Romanian sites

Trascău Mountains

Multiple populations can be found on the limestone massifs of the northern Trascău Mountains: Cheile Turzii, Piatra Secuiului, Piatra Lungă - Vălișoara, Cheile Borzești (Mănoiu & Brînzan 2013). The locality of Cheile Turzii is small in area, but it has probably the biggest population of about 50 to 100 individuals (EEA 2022). In the past this population has been reported to be in danger of disappearing (Floca et al. 1998). The site is important as a lot of other rare species grow there (i.e. *Viola jooi, Aconitum collibotryon, Fritillaria tenella* and others; EEA 2022). This locality is protected as a part Natura 2000 network (ROSCI0035 Romania Cheile Turzii).

Postăvarul Mountains

The species might grow in a protected area of Stejerișul Mare on the slopes of Muntele Tâmpa, however it was only spotted there twice in the last 150 years (1883, 1994) and it is currently unknown if it is still there. The southeastern slope where an island of steppe vegetation appears is the probable location of *D. austriacum* (EEA 2022). Muntele Tâmpa has been a nature reserve since 1962 as well as part of the European Natura 2000 network (ROSCI0120 Muntele Tâmpa).

Ukrainian sites

Ternopil

There are three areas in Ternopil region, where *D. austriacum* grows: Medobory Natural Reserve, its branch Kremenets Mountains and a site near the village Shumsk (GBIF Secretariat 2023). Most frequent and recent sightings (in 2023) come from Medobory reserve, other locations have older data, so the current state is uncertain.

Lviv

All of the populations in Lviv region are located within Northern Podillia National Nature Park (GBIF Secretariat 2023), which has two localities of *D. austriacum*: both in surroundings of the village Stinka, on meadow-steppe slopes of Sypukh mountain and Lisa Gora).

Chmelnyckyj

In the only locality of Podilskyi Tovtry, it was declared extinct due to the creation of limestone quarries, but the species was fairly recently (2019) observed south from village Bila in a very small fragments of dry grassland surrounded by agricultural land (GBIF Secretariat 2023).

Caucasus sites

Dagestan

The populations in Daghestan are located in the northeast part of Caucasus Mountains. There seems to be multiple populations, including ones near villages of Gunib, Rutul, Tekipirkent and Levashi.

Chechnya

Chechnya also has multiple populations. The species grows near village Sintsar, near the lake Ozero Kozenoyam, in the mountain range Armkhi under the ski slope and by the river Ardon.

Karachay-Cherkessia

Quite a few populations seem to be located somewhat near Mount Elbrus: slope of the Gumbashi mountain, north from river Kuban, mountain Ulak, valley of the river Irik, near Kislovodsk village, mountain Malye Sedlo. It is supposed to be quite common there (unofficial information). Some of the populations are situated within Prielbrusye National Park. Other populations within Karachay-cherkessia region, where Elbrus is, include mountain Zakan, near village Teberda, Mashuk mountain near Pyatigorsk, slope of Verhnij Džinal, near the village Aktjube, villages Krasnogorskaya and Žako, west of the city Karachayevsk.

Krasnodar Krai

The sites in Krasnodar Krai include mountain Uzlovaa, valley of the Malaya Laba and mountain Sergiev Gaj.

Armenia

In Armenia, there are also multiple populations, mostly from around the lake Sevan (near villages Shogakat and Lchashen).

GUIDELINES

for species conservation

General guidelines for *Gentiana lutea* conservation

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Gentiana lutea

General guidelines for *Gentiana lutea* conservation

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Introduction

The perennial plant *Gentiana lutea L.* (Fig. 1) grows in mountain or subalpine altitude level of European mountain ranges (Balkans, middle and southern Europe, Pyrenees, Alps, Carpathians) and Minor Asia (Turkey), in Alpine and oro-Temperate and Mediterranean meadows snowed during the winter season, glades of humid pine forests or in rocky and shaded meadows (mainly on calcareous soils). Pharmaceutical and traditional uses have led to the overharvesting of roots, resulting in a decline in the abundance of *Gentiana lutea* across various regions in Europe. Recently, new negative pressures caused by climate change have been added and considered. Despite the non-alarming global status and assessment of this species (Least Concern, LC), the stance is that the species is endangered, and efforts should be made towards its protection and conservation. Included in the CITES convention and European Habitats Directive (92/43/ECC) on a European scale, this species requires crucial measures for its survival. Implementing appropriate actions to mitigate threats and consistently monitoring the population are of utmost importance. Therefore, our endeavors are directed towards presenting suggestions and guidelines in this document for the protection and conservation of this species.

Gentiana lutea L. (photo: Snežana Dragićević)

SCIENTIFIC PART

1. General information about the species

The excessive harvesting of roots due to pharmaceutical and traditional uses has resulted in a decrease in the abundance of *Gentiana lutea* on the territories within its distribution range in Europe (Catorci et al., 2014). This species is categorized as "Least Concern" due to its fragmented distribution and the presence of stable populations in certain regions of its range (European regional assessment, EU 27 regional assessment). As a result, the risk of the species becoming extinct in Europe is relatively low. Nonetheless, it is disconcerting that the species is considered threatened on several national red lists, with the primary threat stemming from the excessive harvesting of this medicinal plant. Especially for the peripheral and isolated populations, mostly on the edge of its southern geographical distribution and ecological niche, climate change, particularly the decrease in winter snow cover and increasing summer temperatures, is another important reported threat. *G. lutea* is presented in the List of endangered medicinal plants in the Annex D to the Council Regulation (EC) No. 338/97 of EU, also on annex V of the Habitats Directive 92/43/EEC, whose purpose is the protection of the plant species by control of their trade.

1.1. Taxonomy

1.1.1. Nomenclature

***Gentiana lutea* L.** (fam. Gentianaceae)

Accepted infraspecifics by POWO (2024) are:

Gentiana lutea var. *aurantiaca* (M.Laínz) M.Laínz
Gentiana lutea subsp. *lutea*
Gentiana lutea subsp. *montserratii* (Vivant ex Greuter) Widler
Gentiana lutea subsp. *symphyandra* Murb.
Gentiana lutea subsp. *vardjanii* Wraber

Four subspecies of *Gentiana lutea* L. are recognised: *Gentiana lutea* L. subsp. *lutea* is distributed all over the range of the species except for the Balkan Peninsula, *Gentiana lutea* L. subsp. *symphyandra* (Murb.) Hayek grows in the Eastern Alps and in the Balkan Peninsula, with small disjunct populations in the Northern Apennines (Rossi et al., 2016a), *Gentiana lutea* L. subsp. *vardjanii* Wraber is endemic in the south-eastern Alps, but its exact distribution is yet to be confirmed, and *Gentiana lutea* L. subsp. *montserratii* (Vivant ex Greuter) Romo is endemic to the Pyrenees.

1.1.2. Life form and strategy

Gentiana lutea L. (commonly known as yellow gentian) is rhizomatous plant, which usually develops one unbranched stout stem (rarely two or three) up to 90 cm tall; it has a basal rosette formed by lanceolate-elliptic leaves measuring 10–30 cm in length and 4–12 cm in width (Prakash et al., 2017). The yellow flowers have a corolla that separates almost to the base, forming 5–7 narrow petals; the ovary is bicarpellate. The fruits are capsules, which hold a great number of elliptic, flattened and winged seeds. Seeds have a linear underdeveloped embryo and show an intermediate complex morphophysiological dormancy (Cuena-Lombraña et al., 2018). The main root can exceed 1 meter in length, weighing up to 7 kg when fresh. This species thrives in grassy alpine and sub-alpine pastures, typically on calcareous soils native to the central and southern European mountains (Knöss, 2018).

Gentiana lutea s.l., a perennial hemicryptophyte and geophyte with rhizomatous characteristics, typically presents a solitary, robust stem ranging from 50 to 120 cm in height, occasionally branching. The plant forms a basal rosette composed of lanceolate-elliptic leaves. Its striking yellow flowers, supported by a bicarpellate ovary, emerge in inflorescences during the summer, reaching heights of up to 120 cm (Fig. 2). These flowers, arranged in pseudo-whorls, boast a corolla divided into five open lobes (Rossi et al., 2016a).

Fig. 2. Gentiana lutea on mt. Rusolija in Montenegro (photo: Snežana Dragićević)

Fig. 3. Gentiana lutea bearing fruit on mt. Sinjajevina, Montenegro (photo: Snežana Dragićević)

Native to the mountainous regions of central and southern Europe, the species thrives at elevations ranging from 800 to 2500 m above sea level. The species exhibits versatile reproductive strategies, employing both vegetative and seed reproduction methods. The plant's capsules, housing numerous elliptic, flattened, and winged seeds, rely on anemochory for dispersal (Fig. 3). The seeds, featuring a linear underdeveloped embryo, exhibit an intermediate complex morphophysiological dormancy.

1.1.3. Variability

The only morphological difference between typical *Gentiana lutea* subsp. lutea and *Gentiana lutea* subsp. symphyandra is in anthers position (*G. lutea* subsp. *lutea* anthers are free whilst *G.lutea* subsp. *symphyandra* anthers are conical forming a tube) and in different stigma appearance after anthesis (*G.lutea* subsp. *lutea* stigma is spirally coiled while *G. lutea* subsp. *symphyandra* stigma is erecto-patent).

1.1.4. Karyology

- Number of chromosomes (2n): 40
- Ploidy level (x): 8
- 2C genome size [Mbp]: 6478.41

- 1Cx monoploid genome size [Mbp]: 809.8
- Genomic content of GC bases: 43.6%

1.1.5. Hybridization

Hybridization in the species *Gentiana lutea* has not been the subject of scientific research so far (there are not any reports on hybrids between taxa). The reproductive success of *G. lutea* strongly depends on pollinators, and there is a partial barrier to hybridization between its varieties (*Gentiana lutea* var. *aurantiaca* and *Gentiana lutea* var. *lutea*).

1.2. Genetics and genomics

Between all subspecies of *Gentiana lutea* agg., the number of chromosomes is 2n = 40. But in some studies on this subspecies variabilities, it is found with Chi-squared test quite significant distictions between *G. lutea* subsp. *vardjanii* and *G. lutea* subsp. *symphyandra* (partitioning of the degrees of freedom) while measuring stigma shapes and bract lengths (Rossi et al., 2016a). *G. lutea* subsp. *montserratii* disticts with specific ovate-elliptic corolla lobes, pollen grains bigger and flower peduncles longer compared to *G. lutea* subsp. *lutea*.

1.3. Species distribution and conservation status

1.3.1. Species area

According to Euro+Med Plantbase (2006+) and POWO (2024), *Gentiana lutea* s.l. is native to Albania, Andora, Austria, Bulgaria, Corse, Croatia, France, Germany, Greece, Italy, Portugal, Romania, Sardegna, Serbia, Slovenia, Spain, Switzerland, Turkey, Ukraine, reported in error in Slovakia and naturalised into Czech Republic. Though it's not noted on Euro+Med Plantbase, *Gentiana lutea* is also present in Bosnia and Herzegovina, Montenegro and North Macedonia (Rohlena, 1942; Đug et al., 2013; Matevski, 2010), and there is some questionable evidence of *G. lutea* presence in Slovakia on GBIF (2024) (Fig. 4).

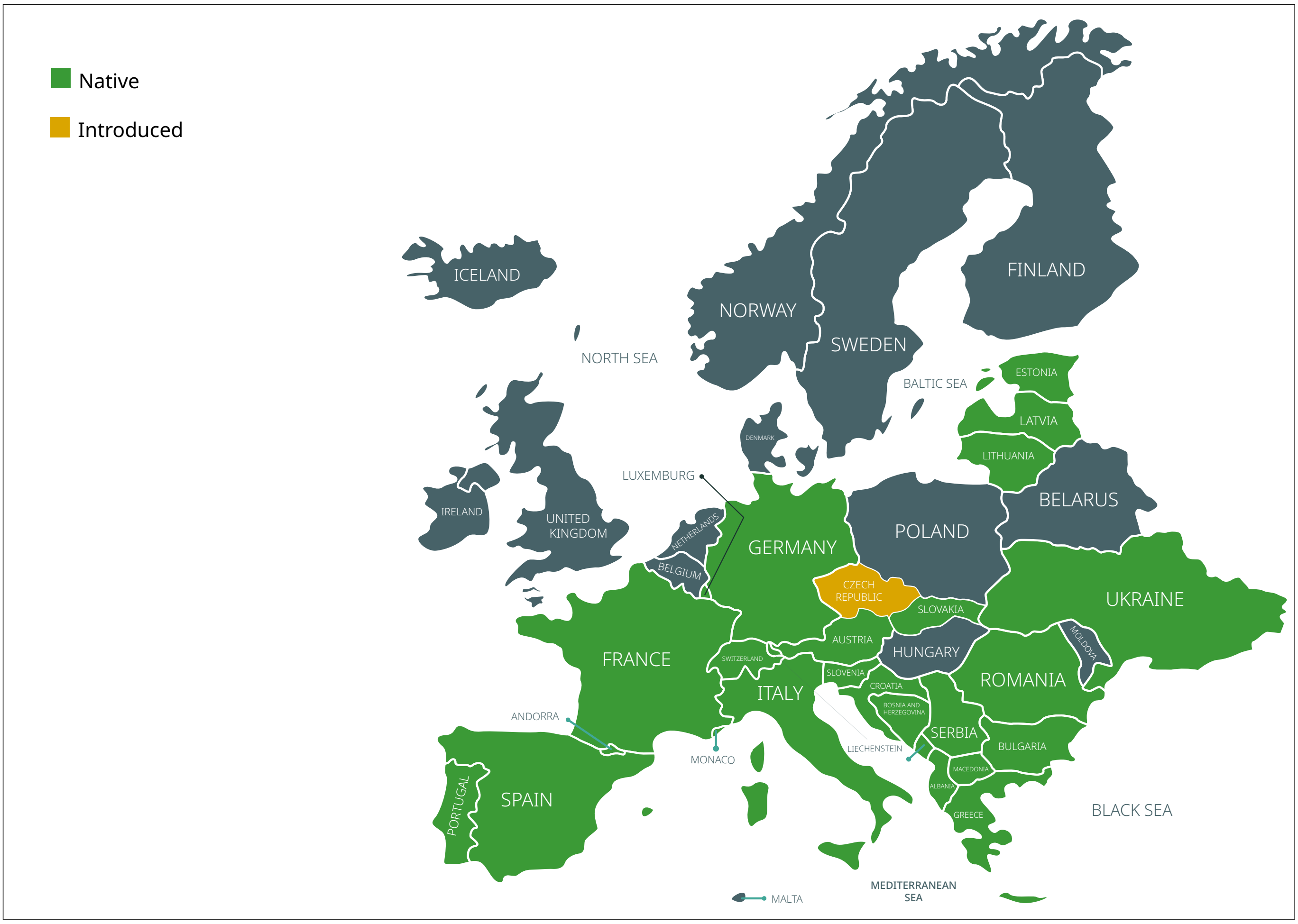
1.3.2. Occurrence, conservation status and threat in particular countries

The excessive harvesting of roots due to pharmaceutical and traditional uses has resulted in a decrease in the abundance of *Gentiana lutea* on the territories within its distribution range in Europe (Catorci et al., 2014). This species is categorized as "Least Concern" due to its fragmented distribution and the presence of stable populations in certain regions of its range (European regional assessment, EU 27 regional assessment). As a result, the risk of the species becoming entirely extinct in Europe is relatively low. Nonetheless, it is disconcerting that the species is considered threatened on several national red lists, with the primary threat stemming from the excessive harvesting of this medicinal plant. According to the IUCN protocol (UCN 2001) *Gentiana lutea* s.l. has been listed as Least Concern (LC) at the European (Bilz et al., 2011) and at Spanish level (Moreno Saiz et al., 2017), Near Threatened (NT) at Italian (Rossi et al., 2016a) and Endangered (EN) at the Sardinian level (Fois et al., 2016), Vulnerable (VU) in Ukraine (Prokopiak et al., 2022) and in Serbia (Bazos et al., 2023), Endangered (EN) in Italy (Fois et al., 2016) and in Croatia (Nikolić et Topić, 2005), while no assessment has been made for Montenegro, Turkey and Greece.

Gentiana lutea is included in Annex V of the Habitats Directive and Annex D to the Council Regulation (EC) No. 338/97 of the EU Wildlife Trade Regulation 318-2008. The regulation covers live specimens of this species, as well as dried and fresh plants, including leaves, roots/rootstock, stems, seeds/spores, bark, and fruits. In several countries, such as Bulgaria, Bosnia and Herzegovina, Albania, Montenegro, Italy, and Slovenia, special collection permits are required from the government. In Serbia and Croatia, the collection of this species is prohibited. It is recommended to replace wild collections by promoting the cultivation of the species, controlling harvesting, and maintaining traditional agriculture.

Gentiana lutea var. aurantiaca (M.Laínz) M.Laínz is the native to Spain. The native range of Gentiana lutea subsp. lutea is European mountains to W. Türkiye. Gentiana lutea subsp. montserratii (Vivant ex Greuter) Widler is the native in N. Spain (Mt. Oroel) and Gentiana lutea subsp. symphyandra Murb. grows from SE Alps to Balkan Peninsula and W.& N. Türkiye (POWO, 2024; Rossi et al., 2016a). Gentiana lutea subsp. symphyandra occurs in Montenegro, according to Rohlena (1942), in various localities: mountain pastures and meadows of Crna planina, Kom, Sinjavina, Durmitor, Javorje planina, Korita rovačka, Žijovo planina, Rogam et Varda, Maglić Pivski, Lukavica planina, Štirni do, Bukovica bellow Mt Kom. Due to the age of data, it is possible that some populations are disturbed because of intensive plant exploitation. According to Vuksanović (2003) and Environmental Protection Agency of Montenegro (2016) it also occurs in localities as Katunina, Jelje, Popov Krš, Ječmen do, Konati and Planinica. This taxa occurs in Bosnia and Herzegovina, it is found at higher altitudes in warm pine forests, on limestone, dolomite and serpentinite. Sometimes they can be found at lower altitudes in oak forests, but they are already quite extinct there (Stefanović, 1977).

Fig. 4. Distribution map of Gentiana lutea s.l. according to literature data



1.4. Biology and ecology

1.4.1. Plant life cycle

Some field surveys suggest that most seeds germinate in early spring. During the flowering season, pollination is facilitated by a diverse range of insects. While several studies have indicated *Gentiana lutea* as self-incompatible, requiring cross-pollination for viable seed production, recent research by Rossi et al. (2016b) suggests partial self-compatibility. One of the reasons is that very often we do not have a population, but only individual plants that are quite far from each other. This implies that, under certain conditions, the plant can produce viable seeds through self-pollination, although optimal seed production still involves the assistance of pollen vectors to ensure successful cross-pollination. Flowering in summer (June-July) and fruiting in late summer (August) (Fig. 5). The wind is the main seed dispersal agent. Corolla has an open structure, which facilitates pollinator access (honeybee, bumblebees, beetles, solitary bees, other Hymenoptera, hoverflies, other Diptera, butterflies, nitidulids) (www.pladias.cz).

Fig. 5. Gentiana lutea bearing fruit on mt. Sinjajevina, Montenegro (photo: Snežana Dragićević)

1.4.2. Phenology

Flowering in summer (June–July) and fruiting in late summer (August). The wind is the main seed dispersal agent.

1.4.3. Life form and strategy

Life form: hemicryptophyte
Life Strategy: C – Competitor
Life strategy (Pierce's method by leaf properties): C/CS
Life strategy (Pierce method, C-score): 77.8%
Life strategy (Pierce method, S-score): 13.3%
Life Strategy (Pierce Method, R-Score): 8.9%

Parasitism and mycoheterotrophy: autotrophic
Symbiotic nitrogen fixation: no nitrogen-fixing symbionts (www.pladias.cz)

1.4.4. Reproduction

1.4.4.1. Generative

Age at first flowering: 3 years

1.4.4.2. Vegetative

G. lutea could also multiply through vegetative propagation: the spreading of rhizomes assures population persistence and growth; hence, even large populations are often represented by few individuals. Vegetatively propagated flowers only after the root system is restored, in 2 or 3 years, during that period it only produces leaf mass.

1.4.5. Habitat

Heliophilous and chionophilus plant, preferably calcicole, also found on siliceous substrates, microthermic and nitrotolerant. It is found in pastures, mountain meadows and mega-meadows, at altitudes between 1000 and 2200 m (Fig. 6). Where the environment is suitable, the species can form dense stands and tends to colonise large areas on abandoned pastures or overgrazed land. Studies conducted in Sardinia (Cuena-Lombraña et al., 2018b), revealed that autumn-winter temperatures and snow days were the main factors in stem development and flowering and subsequent fruit number and seed viability. Seed weight, on the other hand, was mainly determined by temperatures during the flowering period (July).

The communities in which it occurs most frequently belong to the orders *Seslerietalia caeruleae* Br.-Bl. in Br.-Bl. et Jenny 1926*, Seslerietalia tenuifoliae* Horvat 1930 and *Arrhenatheretalia elatioris* Pawl. 1928 or the alliances *Nardion strictae* Br.-Bl. 1926, *Bromion erecti* Koch 1926, *Daphno oleoidis-Juniperion alpinae* Stanisci 1997 or *Juniperion nanae* Br.-Bl. in Br.-Bl., Sissingh, Vlieger 1939 (Gentili et al., 2013).

Fig. 6. Gentiana lutea on mountain meadows of mt. Rusolija, (photo: Snežana Dragićević)

This plant grows in the following Habitats Directive listed habitats
(Commission of the European Communities, 2009):

- **4030 European dry heaths** (EUNIS 2007: F4.2)
- **4060 Alpine and Boreal heaths** (EUNIS2007: F2.21, F2.2A, F2.2B, F2.27, F2.28, F2.24, F2.25, F2.2, F2.23, F2.26, F2.29)
- **4090 Endemic oro-Mediterranean heaths with gorse** (EUNIS2007: F7.4, F7.49, F7.48)
- **5120 Mountain *Cytisus purgans* formations** (EUNIS2007: D3.11)
- **6170 Alpine and subalpine calcareous grasslands** (EUNIS2007: E4.4, E4.41, E4.42)
- **6210 Semi-natural dry grasslands and scrubland facies on calcareous substrates** (*Festuco-Brometalia*) (EUNIS2007: E1.2, E1.22, E1.26, E1.27, E1.28)

- **6230 Species-rich *Nardus* grasslands, on siliceous substrates in mountain areas** (and submountain areas in Continental Europe) (EUNIS2007: E1.7, E1.71, E1.72, E4.3, E4.31)
- **6520 Mountain hay meadows** (EUNIS2007: E2.3, E2.31)

1.4.6. Biotic interactions

1.4.6.1. Herbivory

G. lutea may be considered a toxic species because it contains relatively high levels of herbivory deterrents. But some studies showed that insects consume leaves of each plant during flowering (July). Livestock herbivores consume parts of the plants including leaves, flowers or fruits and parts of the stalk. Livestock was observed feeding on *G. lutea* at the time bearing fruits.

The most common *Gentiana* seed predators are species from Diptera and Coleoptera larvae which are also connected to some parasitoid wasps from the families Ichneumonidae, Braconidae, and Pteromalidae (Kozuharova et al., 2018).

1.4.6.2. Pollination

The main flower visitors of *G. lutea* are bumblebees, cuckoo bumblebees and honeybees and other insects Hymenoptera and Diptera orders (Fig. 7).

In the study of Rossi et al. (2014), Pollinator Performances were quantified. Bumblebees showed the highest fidelity and positive values of Pollinator Performance. Ichneumon wasps and common wasps displayed high fidelity rate and related positive marks of Pollinator Performance. Honeybees were complete of the Passo Lusia population, where they displayed a high rate of fidelity and the highest PoP (Pollinator Performance) index. Ants were found only in one locality, even though they had the biggest population between visitors, their Pollinator Performance was zero, as none of the collected individuals presented more than 5 pollen grains of *G. lutea*.

Fig. 7. Hymenoptera species on *Gentiana lutea* flowers (photo: Snežana Dragićević)

1.4.6.3. Symbiosis

It is known for years that *Gentiana lutea* roots are in endomycorrhizal symbiosis with arbuscular fungi such as *Glomus mosseae* or *Glomus intraradices* (Jacquelinet-Jeanmougin et al., 1987). Fungal subsequent growth is both in intracellular and in intercellular spaces of *Gentiana* roots, while during the penetration it degrades some of the host's wall material.

1.4.6.4. Antagonism

There are some detected Diptera and Coleoptera larvae that are destroying seeds and fruits of *Gentiana* species, and larvae of lycaenid butterflies (such as *Maculinea*) are found only on seeds of *G. cruciata*, *G. pneumonanthe* and *G. asclepiadea* (Kozuharova et al., 2018). Due to further observations, it is discovered that *Gentiana* species develop adaptations by forming specific insecticidal and repellent secondary metabolites (Kozuharova et al., 2023).

It is also discovered that flower color has influence in attraction of insects, for example, both pollinators and seed-predators do prefer yellow flowering individuals over orange colored, but this doesn't give an answer about wide color polymorphism in investigated populations (Veiga et al., 2015).

1.5. Species threats

1.5.1. Current

The traditional use of *Gentiana lutea* includes the following preparations: tincture, fluid extract, dry extract and the comminuted herbal substance for tea preparation by 1) Traditional herbal medicinal product for temporary loss of appetite and 2) Traditional herbal medicinal product for mild dyspeptic/gastrointestinal disorders.

The root and rhizomes of the yellow gentian plant serve as an approved medicinal substance in numerous pharmacopoeias, primarily for addressing mild gastrointestinal issues and promoting appetite, but also the production of alcoholic liquors[1]. Additionally, gentian root finds application in the manufacturing of alcoholic beverages, food items, cosmetics, and anti-smoking products, pharmaceuticals such as anti-inflammatory agents and diuretics. The bitterness of *G. lutea* (European Gentian) is attributed to its principal pharmacologically active compounds, known as secoiridoids (European Medicines Agency, 2018). By the words of Mosula et al. (2014) biggest threats for Ukrainian populations of *Gentiana lutea* are high anthropogenic factor and impossibility for this populations to compete with taller shrubs in those phytocenoses. In Montenegro it is mostly found in alpine and boreal heaths, but can it be also seen on the edges of pine or fir forests. Also this isolation and restriction to marginal habitats is leading to potential local extinctions of border populations (Fois et al., 2016). This last assessment was in accordance with the IUCN Threats Classification Scheme (Version 3.2). The main threats in Sardinia are the excessive root harvesting and grazing, which caused the extinction of the species in some localities. 11.1: Habitat Shifting & Alteration and 6.1 Recreational activities (Fois et al., 2016).

Thus, existing plants are currently impacted by common, known factors: local human harvesting (root harvesting), frequent trampling and grazing by both wild and domestic animals, and habitat loss caused by human activities (e.g. construction of ski pistes and other types of occupation of habitats).

1.5.2. Future

Global warming is causing temperatures to rise in the Mediterranean mountains. This is harmful to the variety of plants and animals in the area and affects how their ecosystems function. In the Mediterranean, where weather patterns change significantly with the seasons, temperature is crucial in determining when plants begin to flower and grow. A vital stage in a plant's life cycle is when its flowers are ready for pollination, which is essential for seed production (Fois et al., 2016).

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1 Plants of the species *Veratrum album* have often been mistaken for *Gentiana lutea*. The main attribute to differentiate between these two genera is that the leaves of *Veratrum* are alternate in contrast to the opposite leaves of *Gentiana*. The medicinal use of Gentianae radix has a very long tradition, while *Veratrum album* is a poisonous plant (Knöss, 2018; Tasić et al., 2001).

Previous studies have shown that it is challenging to predict how rainfall, such as droughts, varying snowmelt timings, and heavy rains, influences the timing of plant flowering. However, for *Gentiana lutea*, the temperature and the duration of snow cover are significant factors (Fois et al., 2016). These conditions vary annually, making it essential to monitor the phenological changes in these plants each year continuously.

The International Union for Conservation of Nature (IUCN) identified climate change as a significant threat (Fois et al., 2016). Comprehensive studies suggest that by 2080, around 30% to 60% of all species could be at risk or extinct. The level of risk differs among various species and depends on their habitats. Plants like *Gentiana lutea*, located in specific, limited areas or at the edges of regions, are particularly vulnerable to climate change.

Indeed, while some past extinctions may have resulted from excessive human harvesting for medical needs, future threats include habitat loss and climate change. Climate-related issues such as water scarcity could soon become a leading cause of species extinction. Besides, other factors, such as species migration, competition with other plants, impacts of historical events, and human activities, as well as the increase of mountain tourism and recreational activities such as hiking, also pose significant risks. These elements could potentially lead to the extinction of certain plants in the near future, as indicated by population predictions from Sardinia (Fois et al., 2016). Additionally, *Gentiana lutea*, like many plants, depends on pollinators for successful reproduction. A decline in pollinator populations due to factors like habitat loss, and climate change could affect its reproductive success (Cuena-Lombraña et al., 2018b). Lastly, the decrease in sexual reproduction, connected with the reduction in genetic diversity within populations, is a significant threat posed by global warming. This has a negative effect on biodiversity conservation, particularly for endangered species like Gentiana lutea.

1.6. Previous implemented management interventions

1.6.1. *In-situ*

The population of *Gentiana lutea* from the Balkans is experiencing a noticeable decline, particularly in Bosnia, Albania, and Montenegro. In contrast, populations in other European countries remain stable, as they are not subject to wild collection. Over the past 15 years, the Balkan population of this plant has decreased by approximately 30%, underscoring the importance of conservation efforts.

According to our knowledge, two conservation projects have been conducted for this species, one in Montenegro and the other in Italy. In Montenegro, a plantation was established using seeds collected in natural conditions Bjelasica Mts, Sinjajevina Mts), and plants from this plantation were reintroduced into their natural habitat (habitats of surrounding nursery mountains). The planted plants have successfully grown in the wild (the project is carried out by NVO Natura from Kolašin). In Italy, seeds of *G. lutea* ssp. *lutea* from all naturally occurring populations were collected and their genetic material was preserved at the Sardinian Germplasm Bank (BGSAR). Seeds of *G. lutea* subsp. *lutea* were collected from the largest locality present in Sardinia in order to maximize the genetic diversity of the material. Successively, plants obtained from seeds were cultivated for 1–3 years in the greenhouses of the Agenzia FoReSTAS (Agenzia Forestale Regionale per lo Sviluppo del Territorio e l'Ambiente della Sardegna, Autonomous Region of Sardinia), located in the municipality of Talana, close to the selected area. Before performing the translocation, the selected area was fenced following the previous experiences in Sardinia. The translocation has been carried out in two periods: the first in autumn (December 2014) and the second in spring (March 2015) by using plants of different ages (100 plant of 1 year old and 100 plants of 3 years old). (Cogoni et al., 2018).

Analyzing populations in Sardinia, Fois et al. (2016) heartily recommend in situ activities, such as monitoring, fence protections and translocations as the priority. Conducting local research at all known locations, collecting the most significant data and analyzing them according to the ecological characteristics of microlocations, are the initial step in the process of establishing and implementing activities in the conservation and improvement of the status of the species. Also considering that regional analyses should be carried out by local researchers who are supposedly the best experts in their own territory, those practices are in many cases a very effective tool for the conservation management of biodiversity (Fois et al., 2016).

The methodological framework for conservation measures, guided by assessments at the regional level as proposed by Fois et al. (2016), provides a solid foundation for articulating the most effective theoretical conservation measures that can be suggested for each individual case (Table 1).

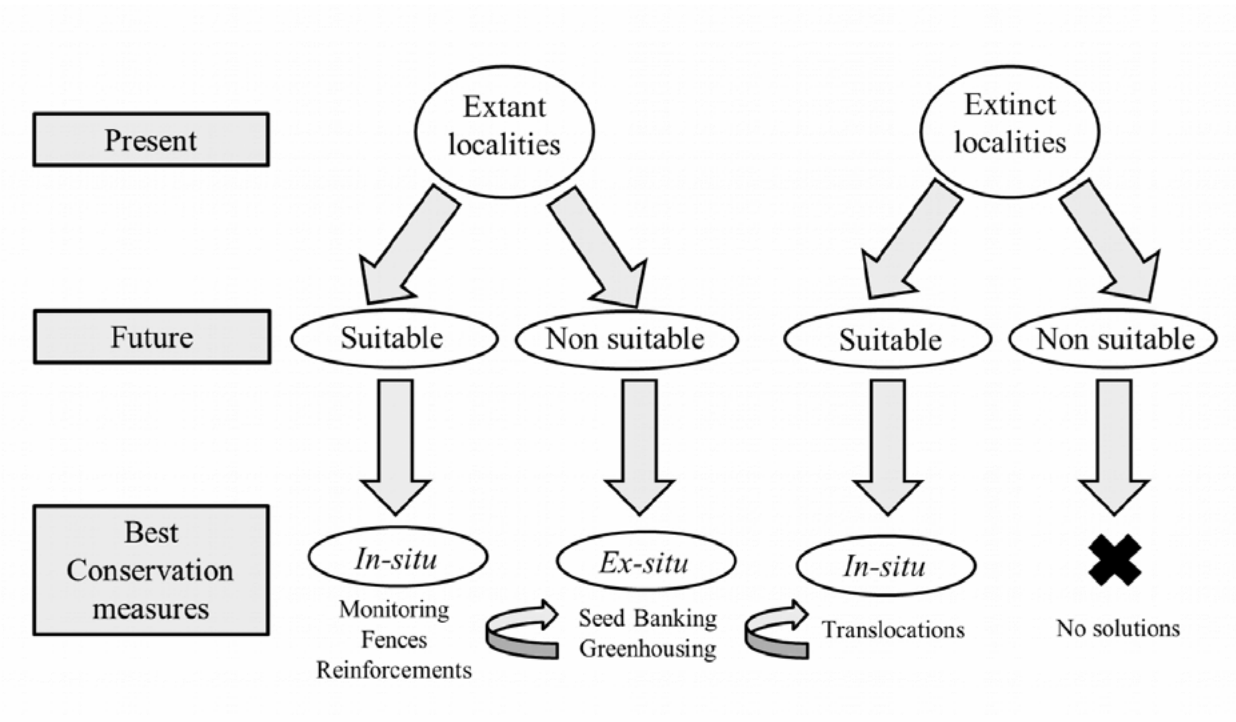

Table 1. Methodological chart of the conservation measures for endangered populations, guided by assessments at regional level. (Fois et al., 2016)

1.6.2. Ex-situ

Seed banking

Gentiana lutea seeds can survive in conditions preserved in seed banks (desiccation, low temperature). In accordance with the European Network for the Conservation of Wild Plants (ENSCO-NET), seed conservation activities for *Gentiana lutea* are taking place in the following countries: Greece, Germany, Poland, Belgium, Austria, Portugal, Spain, Italy, Cyprus, Switzerland, Finland, Luxembourg, France, Portugal, Cyprus, UK, Slovak Republic, Romania, Ireland, Norway, Czech Republic (37 institutions) Seeds were collected in the following countries: Spain, Slovenia, Switzerland, Austria, Italy, Greece, France (http://enscobase.maich.gr/fullnamelistacc.tml?taxid=4121419&orderby=accession_id&dir=asc, 29. October 2023)

Multiplication

Recent research has shown that the seeds of *G. lutea* are characterized by medium complex morphophysiological dormancy and that temperature is a critical environmental factor for germination (Cuena-Lombraña et al. 2018a).

Seeds from various sizes of localities exhibited noteworthy physiological variability in terms of their final germination percentages. This variability holds significance for both conservation efforts and the germination of seeds from diverse sources. When devising a conservation strategy that involves the *ex situ* cultivation of plants, it is crucial to take into account the optimal germination procedures for isolated populations (the decrease in seed germination noticed in smaller local areas suggests the importance of heightened focus on and surveillance of these small populations, owing to their heightened susceptibility to extinction). Such an approach could effectively help alleviate the stress on wild populations caused by root harvesting.

2. Aims of rescue plans

The aim of the regional action plan is to preserve the species *Gentiana lutea* in locations where this species still exists, but also to return the species to locations where it was once present but was destroyed or brought to the brink of extinction due to excessive exploitation due to uprooting, but where its habitats are preserved. To define broader objectives related to rescue actions that encompass all time periods, it is crucial to have a good understanding of the species' biology, locally analyzed populations, and on-the-ground situations, as well as the present and potential pressures in the present/future with an assessment of the significance of the impact and the period of action.

Short-term

Specific goals and measures planned for implementation in the upcoming months or years to promptly tackle pressing challenges in the conservation of *Gentiana lutea* are: protection of species and all localities on which grows tall immediately; prohibition of collection of the roots; habitat management: preventing habitat overgrowth competeing species; identification of the total number of individuals based on molecular analyses; research of genetic variability; finding new populations; monitoring of defined parameters of all known and new populations in all localities; informing managers and decision-makers.

Mid-term

Goals and actions to be undertaken in the medium time frame for achieving sustainable recovery of the populations of *Gentiana lutea* are: habitat management - preventing habitat overgrowth competing species, finding new populations, research on the structure, variability and changes (succession) of habitats, study of pollination and reproductive success of the species and dissemination of information through scientific and professional publications, the internet, and others.

Long-term

In order to ensure the permanent survival of the plant and its habitat in the future, it is necessary to define long-term strategies that have these goals in focus for *Gentiana lutea* which, among other things, provides for the purchase of land if the habitats are privately owned. Taking into account that seeds of *G. lutea* are characterized by intermediate complex MPD and that temperature is a critical environmental factor for germination to occur. This information is particularly relevant to conservation of this species also considering the threat represented by global warming, as its distribution in the southern part of Europe, that mainly regards the upper sectors of mountains. The necessity of programming management actions such as translocation programmes and population reinforcements in order to help preserve high genetic diversity of this plant of European interest and of high economic value.

3. Suggestion of interventions

3.1. Site management

3.1.1. Tree cutting

Yellow gentian grows in mountainous regions, on meadows and open slopes. The expansion of woody species in meadows will lead to changes in the ecological conditions of the habitat, as the tree canopies can block sunlight and create cool shadows. This can reduce the growth of plants in the meadow that require ample sunlight, such as *Gentiana lutea*. Additionally, woody species compete for water and nutrients. This can result in resource depletion for meadow plants and a reduction in vegetation density. In any case, woody species pose competition for *Gentiana lutea* and should be removed from their habitats.

3.1.2. Mowing

Mowing could reduce competition and enhance ground-level light availability, facilitating the spread of the species, but impact has not been proven in practice. However, mowing also signifies the presence of people who continue to harvest the roots of this plant in the Balkans, leading to its destruction in the wild. In Montenegro in locations where high-altitude pastures are observed, which are not mown but grazed by livestock, the plants thrive, with these areas being distant from villages, settlements, and even mountain huts. Germination and seedling establishment are typically better in places where the vegetation is not excessively trimmed during mowing, creating a humid microclimate that prevents soil desiccation.

3.1.3. Burning

The habitats of *Gentiana lutea* are high-altitude meadow and pasture environments. These habitats can be threatened by wildfires if they are small and surrounded by burning forests. Such adverse impacts can have a detrimental effect on the biodiversity of meadows and pastures where this species grows. As it belongs to perennial plants with a developed root system, smaller ground fires have no effect because it easily regenerates vegetatively, but in those situations when the fire occurs in late autumn, the seeds are damaged. A more detailed description of the impacts is not known because such situations have not been recorded in Montenegro, where this plant grows at higher elevations, above 1000 meters, where the conditions are colder and more humid (it does not stand high temperature and needs high precipitation).

3.1.4. Grazing

In accordance with the IUCN classification scheme, threats have been ranked as follows: Threat 5.2.1, the intentional gathering of terrestrial plants for various uses (historical and current information); Threats 2.3.1 and 2.3.2, nomadic and small-scale grazing, ranching, or farming, which have had only a marginal impact on the regional conservation status of *G. lutea*. In Montenegro, grazing was not recognized as a threat. But in Sardinia one of the main threats was overgrazing – especially of reproductive parts – livestock overtrampling, which caused excessive soil erosion, significant impacts on reproduction and the successive extinction of the species in certain localities. A problem that can also threaten this forest is natural succession. As this is a type of open habitat that requires full enjoyment of the light, where there is no livestock keeping that suppresses the aggressive dendroflora, the meadows are quickly overgrown with dendroflora and the habitat is lost.

3.1.5. Removal of topsoil layer

The removal of the topsoil layer can have significant detrimental impacts on plants. Topsoil is the most fertile, and contains organic matter, nutrients, and microorganisms that are crucial for plant growth. Also, there is reduced water retention, altered soil structure, increased vulnerability to erosion, impact on soil pH, etc. Information about the influence of this effect on *Gentiana lutea* is not known from literature and practice.

It should also be emphasized here that it grows only on those parts of shallow high mountain soils where it can provide roots in rock crevices that are filled with humus.

3.1.6. Fencing

In nature, plants can "fortify" themselves for protection and survival for various reasons, including defense against herbivorous animals. However, in the case of *G. lutea*, a barrier would be necessary when transplanting young seedlings into natural conditions, as was done in Sardinia as part of the project in Talana. The site was fenced off before the translocation. A total of 200 individual plants were translocated, with each plant being carefully labeled for future monitoring activities. Subsequently, two additional fences were constructed in a different location near the translocation site, and another 200 plants were translocated there.

3.1.7. Reduction of flower harvesting

The yellow gentian is highly valued for its medicinal qualities, primarily due to the strong bitterness found in its root, making it a key ingredient in homeopathic treatments. The roots contain abundant bitter glycosides, such as gentiopikrin and amarogencine, which are also used in making liqueurs. Nonetheless, commercial exploitation of wild populations for these purposes has contributed to its decline in certain regions. Furthermore, traditional methods of harvesting gentian roots require long recovery times for these wild populations (Cuena-Lombraña, 2016).

Thus, after pruning, where a part of the roots is left in the soil for restoration, and when the plant grows, it cannot flower for the next few years until it properly restores the root system, during which time it produces only ground leaf mass (personal observations).

3.1.8. Liming

The characteristics of the local environment, including geographic coordinates (longitude and latitude) and exposure, together with the advantages and limitations of climatic and soil conditions are important factors. Conditions like low annual precipitation and high summer temperatures cause bad gentian growth. Also, the soils being well supplied in organic matter and medium supplied in potassium, with the pH mostly ranging from medium to strong acid, proved to be suitable for gentian growth. Otherwise, at lower altitudes it grows on all exposures, regardless of soil type, while at high altitudes it avoids northern ones.

3.2. Species targeted interventions

According Bazos et al. (2023) various measures are in place at the national level in different countries, including:

- **France**: Listed in la liste des espèces végétales protégées en région Champagne-Ardenne (21) and identified as a protected species in seven Départements (Alpes-Maritimes, Haute-Corse, Alpes-de-Haute-de-Provence, Jura, Corse-du-Sud, Corse-du-Sud, Haute-Corse).
- **Germany**: Classified as Vulnerable (level 3) on the national red list (1996) and under national protection.
- **Greece**: Protected under Presidential Decree 67/81, with presence in seven Natura 2000 sites.
- **Italy**: Partial inclusion in parks and Natura 2000 sites. Protected by Regional laws in Liguria, Lombardia, Emilia-Romagna, Umbria, Molise and Calabria. A project to recover the population was done in Sardinia (2015-2018).
- **Slovenia**: Formerly protected by law and listed as Vulnerable in the national red list.
- **Serbia**: Estimated to be Vulnerable.
- **Switzerland**: Classified as Least Concern in the national red list (Moser et al., 2002) with partial inclusion in protected areas.
- **Ukraine**: Included in the Red Data Book as Vulnerable, and protected in the Carpathian reserve, Carpathian national park, in Apchineckij zakaznik.
- **Montenegro**: Protected by national law from 2006.
- **Bosnia and Herzegovina**: It is on the list of endangered species and its collection is prohibited

3.2.1. Ex-situ conservation

3.2.1.1. In-vitro culture

Micropropagation of *Gentiana lutea* is carried out by proliferation of axillary shoots initiated on nodal stem segments excised from seedlings germinated *in vitro* (Momčilović et al., 1997). The culture medium used was MS (Murashige & Skoog, 1962) with 3 g l^{-1} sucrose, and 7 g l^{-1} agar. Higher shoot proliferation was achieved by adding 2.28 μM indole-3-acetic acid (IAA) and 8.88 μM 6-benzylaminopurine (BAP). Micropropagated shoots were rooted by culture in MS medium supplemented with 10.74 μM α-naphtaleneacetic acid (NAA) for two weeks, followed by transfer of rooted plantlets to plant growth regulator-free MS medium for one month.

Alternatively, Petrova et al. (2019) multiplied *in vitro Gentiana lutea* shoots by culturing nodal segments from aseptically obtained seedlings in MS medium with 2 mg l-1 zeatin and 0.2 mg l^{-1} IAA. *In vitro* shoots were rooted in MS medium with 1 mg l^{-1} silver nitrate and 1.0 g l^{-1} IBA.

Gentiana lutea has also been regenerated by indirect adventitious organogenesis (Yildirim, 2019). Organogenic callus was initiated from cotyledon nodes excised from *in vitro* germinated seedlings. Higher callus induction percentage was obtained on MS medium supplemented with 1.135 μM TDZ and 4.90 μM IBA. Although higher shoot regeneration percentage was achieved on MS medium only containing 9.08 μM TDZ, maximum number of shoots per explant was found in MS medium with 1.135 μM TDZ and 2.450 μM IBA. Regenerated shoots were rooted on MS medium supplemented with 30 g l^{-1} sucrose, 5.37 μM NAA, 5 g l^{-1} activated charcoal gelled with 7 g l^{-1} agar. Cultures were incubated at 24 ± 1ºC under a 16 h light photoperiod and an irradiance of 35 μmol m^{-2} s^{-1}.

Somatic embryogenesis has been used in *Gentiana lutea* for synthetic seed production and medium-term conservation (Holobuic & Catana, 2012). Embryogenic cultures were initiated from hypocotyl segments and roots derived from aseptically germinated seedlings. Embryogenic tissues obtained were used as initial explants in a second cycle, which gave rise to increased embryos production.

For synthetic seed production, somatic embryos 1–2 mm in size, isolated or forming small aggregates were encapsulated in alginated beads using the same culture medium without plant growth regulators. Synthetic seeds were cultured on MS medium with nutrients at half strength and maintained at 10ºC under light conditions.

3.2.1.2. Seed and pollen banks

Seed collections in relict populations are therefore recommended in order to capture as much of the genetic variability of the taxon as possible across its distribution area. Certain experiments have underscored significant physiological variations among seeds from various growth locations. It is crucial to consider these distinctions when devising ex situ conservation strategies for this species. This underscores the significance of harvesting and safeguarding seeds from diverse sources to enhance the genetic diversity within seed collections stored in germplasm repositories.

According to BGCI, *Gentiana lutea* L. is deposited in 16 gardens (https://plantsearch.bgci.org/). Cuena-Lombraña et al. (2016) had developed germination tests under controlled and natural conditions and demonstrated the significance of variations in seed behavior among different accessions of the same taxon. The experiments underscored notable physiological distinctions among seeds from various growing sites, evident in the final seed germination percentages observed under controlled and natural conditions. These variations are crucial considerations for ex situ conservation strategies for this taxon, underscoring the necessity of collecting and conserving seeds from diverse origins to enhance the genetic diversity of seed collections stored in germplasm repositories. Additionally, insights into seed germination hold significant economic potential.

3.2.1.3. Plant cultivation

Based on cultivation guidelines, it is advised to address seed dormancy by either treating the seeds with gibberellic acid or subjecting them to two months of cold stratification at 2°C. Plantations can be established in both autumn and spring, with varying planting densities ranging from 5 to 10 plants per square meter. After the initial two to three years of growth, measures like fertilization, weed control, and irrigation become less demanding. Ideally, the cultivation period should span four to six years to achieve economically viable root yields.

According to Bosnia and Herzegovina's experiences in the cultivation and production of "lincura", sowing is done in autumn, and the seeds are left to natural processes from autumn to spring. Unfortunately, some of the seeds perish in such a treatment, but about 15 plants are obtained. Sowing is done directly in containers. Otherwise, seeds are collected from plants in nature.

3.2.2. Plant translocation

3.2.2.1. Population reinforcement

Population reinforcement or enhancement of *Gentiana lutea* could involve strategies to protect, propagate, and increase the population of this plant in its natural habitat. There are some general strategies that can be employed for population reinforcement while specific strategies may vary based on the ecological characteristics of the region where *G. lutea* grows. Conservation efforts often require collaboration between many organizations like government agencies, conservation organizations, local communities, and researchers.

There are some activities that can be included in general strategies for population reinforcement of any plant species, including *G. lutea*:

Habitat protection

- Identify and protect the natural habitats where *Gentiana lutea* grows.
- Implement conservation measures to prevent habitat destruction, such as deforestation or urbanization.

- Implement preventive fire protection measures
- Implement measures to stop succession in its habitats, with active conservation

Monitoring and research

- Conduct regular monitoring to assess the population status and health of *G. lutea*.
- Support research initiatives to better understand the ecological requirements and life cycle of the plant.

Propagation and cultivation

- Establish cultivation programs to grow G. lutea in controlled environments.
- Develop techniques for successful propagation, including seed germination and vegetative propagation.

Reintroduction programs

- Consider reintroduction programs in areas where the population has declined.
- Select appropriate sites based on ecological conditions and conduct careful planning to ensure successful reintroduction.

Community involvement

- Involve local communities in conservation efforts.
- Raise awareness about the importance of *G. lutea* and the need for its conservation.

Genetic diversity conservation

- Implement measures to maintain genetic diversity within populations.
- Avoid overreliance on a limited number of individuals for propagation.

Legal protection

- Advocate for and implement legal protection measures for *G. lutea,* ensuring that harvesting and trade are sustainable and regulated.

The cultivation of *G. lutea* is greatly affected by the ecological conditions of its habitat. Franz (2012, as cited in Radanović et al., 2014) noted its successful growth in a wide range from central Finland (200m above sea level) across western France and Bavaria (500–1200m above sea level) to the Apennines and Pyrenees in Southern Europe

(1600m above sea level). The plant adapts well to various climates, including alpine, Mediterranean, and mountainous regions. It flourishes in areas that experience clear seasonal changes, have cold winters, and receive over 750mm of rain annually.

Soil suitable for *G. lutea* should be deep, without stones, and able to hold air and water well (Stepanović & Radanović, 2011, as cited in Radanović et al., 2014). The soil should be slightly acidic (pH below 6.5) with a sandy-loam texture and low to medium humus level. Therefore, alkaline soils (pH 7–8) should be avoided due to the potential for iron (Fe) chlorosis in some *G. lutea* ecotypes. Research in both the Balkans and Italy, commonly found the soil to have low phosphorus and moderate potassium levels.

G. lutea is cultivated as a perennial plant, staying in the soil for a minimum of five years (Radanović et al., 2014). For example, it is frequently planted in Serbia on deteriorating natural meadows. The practice of crop rotation is advised, starting with buckwheat or oats and followed by grains or legumes, while replanting *Gentiana* on the same plot is not recommended. Phosphorus supplementation in the soil shows limited effectiveness due to the low mobility of phosphates. In Serbian trials on acidic soils, iron chlorosis was not a problem, in contrast to Italy, where less emphasis was placed on fertilization (Radanović et al., 2014).

Proper irrigation immediately after planting is vital, especially during droughts, and remains essential in the initial stages of vegetation, i.e., from May to July, in order to counteract soil dryness. After the first year, managing yellow gentian becomes easier, with weed control, fertilization, and irrigation being the primary care practices until the end of the growth period. Past cultivation experiences in Southern and South-eastern Europe have identified climatic conditions and seedlings' slow growth and rooting in the first two years as significant obstacles, resulting in considerable plant loss and reduced harvests per hectare (Radanović et al., 2014).

In summary, successful *G. lutea* cultivation hinges on selecting optimal sites, considering soil, climate, and exposure, and implementing strategies to control perennial weeds, such as choosing suitable pre-crops and using both mechanical and chemical treatments (Radanović et al., 2014). Using high-quality nursery plants and seeds, along with having the right equipment for soil care and systems for watering, is essential for a successful harvest. Furthermore, research aimed at understanding the variations in root quality among different *G. lutea* ecotypes and enhancing nursery plant production and nutrition models, with a focus on phosphorus, is vital for meeting the specific needs and improving cultivation practices in South-eastern Europe (Radanović et al., 2014).

3.2.2.2. Reintroduction

In the report of Cogoni et al. (2018) translocation has been carried out after managing an ecological study and analysis of historical and current natural distribution ranges of *G.lutea* in Sardinia. They determined series of goals for right translocation and reintroduction of *G.lutea*: Reintroducing plants in settings where species was lately extinct due to peculiar causes; Determining the circumstances essential for *G.lutea* reintroduction to be on velvet; making the results of this project at hand for future herb reintroduction experiments; examining how successful plant renewing has been in establishing possible, self-sustaining population in Sardinia and in the Mediterranean.

Similar measures and guidelines were applied to the *G. lutea* in Ukraine. According to Prokopiak et al. (2022), three studied populations of *G. lutea* in the Ukrainian Carpathians were found to be unstable, with two being relatively stable and only one exhibiting stability. The uneven distribution of these populations is attributed to anthropogenic impacts such as the harvesting of raw materials, grazing, and recreational activities, as well as biotic factors like the low plasticity of the species and overgrowth of shrub populations. Consequently, there is a recognized need for their restoration. One of some measures that are preserved for restoring natural reserves of *G. lutea*, include introducing or reintroducing *G. lutea* through the creation of artificial plantations in natural habitats. This approach aims to maintain genetic diversity and increase the number of habitats supporting this species. Create introduced intermediate populations to preserve genetic diversity, addressing the significant differentiation observed among *G. lutea* populations.

3.2.2.3. Assisted colonization

Conservation translocation involves intentionally moving living organisms for conservation, comprising reinforcement, reintroduction, or introduction plans. Its goal is to establish resilient populations, enhance species survival, and improve genetic diversity. This can include increasing numbers in small populations, reintroducing individuals, or establishing new safe locations. Recently, it encompasses assisted colonization for climate change adaptation, supporting range shifts. Translocations are recognized as crucial tools to prevent plant extinctions, conserve threatened species, and address climate change effects (Fenu et al., 2023).

Assisted translocation programs for *Gentiana lutea* have been implemented in regions where the plant naturally thrives but has suffered a decline or destruction due to excessive and uncontrolled exploitation. However, there is currently no information indicating that these initiatives specifically target overcoming barriers imposed by climate change.

3.2.2.4. Ecological replacement

There is no available data on the practical application of ecological substitution/replacement as a conservation strategy for Gentiana lutea. For this reason, the conditions of breeders should be analyzed and compared with the conditions of natural habitats

3.2.4. Suitable approach for plant translocation

Sowing

G. lutea is a species of high economic importance. According Cuena-Lombraña (2016) the optimal germination protocol for this species consists of a period (ranging from one to three months) of cold stratification at ca. 0°C in dark conditions (to break physiological dormancy), followed by seed incubation at 10-20°C under photoperiod conditions of 12/12 hours.

Based on cultivation guidelines, it is advised to address seed dormancy by either treating the seeds with gibberellic acid or subjecting them to two months of cold stratification at 2°C. Plantations can be established in both autumn and spring, with varying planting densities ranging from 5 to 10 plants per square meter. After the initial two to three years of growth, measures like fertilization, weed control, and irrigation become less demanding. Ideally, the cultivation period should span four to six years to achieve economically viable root yields.

Transplanting of new individuals

In the case of Sardinia translocation, were following the post-planting monitoring: All transplanted plants were monthly monitored from April to September recording plant growth and survival rate; flowering and reproduction of the established plants and number of new established seedlings are planned to be monitored after five years from the transplanting action. Management actions aimed to reduce the natural vegetation evolution has been periodically carried out by removing the fast-growing species in the site (e.g. *Erica arborea* L., *Rubus ulmifolius* Schott., etc.). Preliminary results indicated a similar survival rate both for ages of plants and seasons of translocation. The higher mortality rate was observed during the first year, while this rate diminished up to zero in the next years. After three years, the survival rate was sufficiently high, with 94 plants alive (47%) (Cogoni et al., 2018).

Support of pollination

Due to an open structure of corolla (Fig. 8), pollinator access is facilitated to flower nectaries (main pollinators are from Hymenoptera and Diptera orders) (Cuena-Lombraña, 2016). The disruption of pollinator-plant mutualisms in small populations (Rossi et al., 2014) might play an important role in the reduction of the germination ability in the population. In their study, distinction was made between dynamic and sedentary pollinators. In some cases, most species carried pollen while touching receptive stigmas. Nevertheless, dynamic pollinators visit flowers of the identical plant and then fly on other herbs, making efficient cross-pollen movement. Conversely, sedentary pollinators like beetles, flies etc., are more contributing to geitonogamy, making several visits within the same pseudo-whorls, and expanding intra-plant pollination.

Fig. 8. Open structure of corolla in *Gentiana lutea* (photo: Snežana Dragićević)

4. Monitoring of impact of interventions

According to Prokopiak et al. (2022), establishment of a monitoring system for *G. lutea* populations is essential for studying population dynamics and predicting their development over time. Three studied populations of *G. lutea* in the Ukrainian Carpathians were found to be unstable, with two being relatively stable and only one exhibiting stability. The uneven distribution of these populations is attributed to anthropogenic impacts such as the harvesting of raw materials, grazing, and recreational activities, as well as biotic factors like the low plasticity of the species and overgrowth of shrub populations. Consequently, there is a recognized need for their restoration. To preserve and restore natural reserves of G. lutea, they proposed the following measures:

Implement regulations to mitigate the impact of human activities, particularly by limiting cattle grazing, to safeguard the natural habitats of unstable populations.

Introduce or reintroduce *G. lutea* through the creation of artificial plantations in natural habitats. This approach aims to maintain genetic diversity and increase the number of habitats supporting this species.

Create introduced intermediate populations to preserve genetic diversity, addressing the significant differentiation observed among G. lutea populations.

4.1. Plant populations – genetic and biodiversity monitoring

It is necessary to conduct a detailed investigation of the demographics of populations, with a focus on growth dynamics, reproduction, and survival as well as analyze genetics, particularly monitoring genetic diversity within *G. lutea* populations. Information about gene flow can provide a deeper understanding of evolutionary processes and potential threats.

Studying the genetic and diversity aspects of *Gentiana lutea* will aid in assessing the health and conservation of this species. Define a use of appropriate methods to monitor the genetic structure of populations and identify key factors influencing their stability. Also are important the implications of these findings for the long-term sustainability and management of this species.

Dettori et al. (2018) were investigating a study on genetic structure and diversity of *Gentiana lutea*, and with ANOVA analysis they discovered the amount and pattern of genetic variation in 13 subpopulations in Sardinia. Using amplified fragment length polymorphism (AFLP) they found high genetic diversity, with tendention to decrease in smaller subpopulations, and weak genetic structure in these subpopulations. Their recommendation for conserving these subpopulations is neccessarity to plan *ex situ* and *in situ* management actions, like long-term preservings of its seeds in germplasm repositories, while reinforcing and monitoring their populations.

4.2. Site quality

The quality of the habitat is of great importance for the life of *Gentiana lutea*. This complex of characteristics includes all environmental factors in a certain area that affect the growth, development and survival of plants. These components, both abiotic and biotic factors, together make "site quality" as the key factor that determines the success of plants in a particular location. Protecting and conserving habitats and its conditions are essential for the preservation of populations of *G. lutea*. In populations that are small and in decline it is noticed that plants produce fewer seeds per fruit and per plant than in the larger and more stable populations (Kéry et al., 2000). So these endangered populations may face a greater short-term risk of extinction because of decreased reproduction, and a greater long-term risk because they are much less capable of countering environmental changes.

In considering the importance of habitat quality, the factors to focus on are of abiotic and biotic nature. The abiotic data that need to be collected and analyzed, especially in areas where there is no information on *G. lutea* (such as the Balkans), are as follows: **soil** – soil composition, its structure, and characteristics such as pH values, organic content, soil texture, and its water retention capacity; **microclimatic conditions** – specific microclimatic conditions at the location, including sunlight, temperature, air humidity, and wind; **hydrological conditions** – water availability at the location, including precipitation amounts, types of watercourses, and the soil's ability to retain water; **topographic characteristics** – relief, slope, gradient and orientation of slopes that can impact ecological conditions at the location. Biotic variables encompass: **pollinators –** variety and performances; **competition with other species** – competition for light, water, and nutrients; **presence of predators or diseases** – identification of potential threats from herbivores, parasites, or diseases that may affect the *Gentiana lutea* population; **anthropogenic influences** – analysis of the impact of human activities at the location, including factors such as urban expansion, agriculture, forestry activities, and other human influences.

4.3. Ex-situ

Ex situ measures, crucial for preventing immediate extinction, include experimental examinations of germination timing in natural sites, understanding seed behavior in soil, and investigating germination responses under laboratory conditions. if genetic and morphological differences in populations, especially due to smaller sizes and greater distances from central populations, it is vital to assess the impact on seed ecophysiology and germination in these spatially isolated groups. Prior research indicates that germination in many mountain plants is influenced by cold-wet stratification cycles, releasing seed dormancy in transient and permanent seed banks. Dormancy plays a pivotal role in optimizing germination success by controlling timing. However, such data is scarce, especially in the Mediterranean mountains. Our research addresses this gap by contributing to the conservation of *Gentiana lutea*, suggesting optimal germination and multiplication protocols, and providing insights into various seed dormancy types. This information is crucial for understanding evolutionary relationships and natural selection, favoring germination patterns that minimize risks during seedling establishment in adverse environmental conditions (Cuena-Lombraña, 2016).

In developing models for the *ex situ* conservation of *G. lutea* for all taxa, it is necessary to examine the traits on which the success of conservation actions will depend, such as: seed germination ecophysiology and the influence of growing sites on seed germination; seed germination time in the soil by seed burial experiments; the class and the type of seed dormancy and relate the dormancy breaking through embryo growth and radicle emergence; the thermal requirements for seed germination (optimal protocol of germination). Namely, the experiments conducted by Cuena-Lombraña (2016) on materials from Spain and Italy (N-Spain, CE-Sardinia) have revealed substantial physiological differences among seeds from various growing sites, evident in final germination percentages under controlled and natural conditions. These distinctions underscore the need to consider these factors when designing ex situ conservation strategies for this taxon. They also highlight the importance of collecting and preserving seeds from diverse origins to maximize genetic diversity in germplasm repositories.

Moreover, the knowledge gained about seed germination has significant practical implications. Understanding the factors influencing germination timing aids in planning effective propagation strategies, especially for threatened or economically important plants (Cuena-Lombraña, 2016).

Stability and reproduction species of Gentiana in general in *ex situ* collections depend on ombro and thermal-regimes, chemical constitution of soil, intensity of solar radiation and light spectral composition to encounter the essential needs of taxons. If the refered factors are inappropriate for the physiological and ecological needs of the species, it is tough to get feasible assembly of plants *ex situ* (Hrytsak et al., 2021).

5. Further research

Although this species relishes international and national protections, we must conduct comprehensive research on it, especially in areas without significant data, like the Balkans. For example, in Montenegro some data has been gathered in recent years for the Natura 2000 network mapping, but the present distribution remains unknown. Notably, the species has suffered a decline due to exploitation — specifically, the excavation of its underground components for medicinal purposes. The lack of effective control and response from authorities has left the species unprotected in its natural habitat in the true sense of the word and made it protected only on paper. Considering these circumstances, urgent research is imperative to assess the current situation, focusing on both the distribution and abundance of the species. Subsequently, competent institutions must, in accordance with existing laws, initiate conservation measures and implement a rescue plan aimed at restoring specific habitats and populations.

6. Public awareness

For this species, it is extremely important to preserve the openness of the habitat and monitor the growth of that habitat, as well as risk factors. One of the main factors endangering this species in the Balkans is the massive digging up of the roots of this plant and its use for medicinal purposes. It is crucial to monitor and sanction such practices. as well as to raise awareness about this topic via workshops, public campaigns, etc. The commitment of volunteers is particularly important when establishing regular management or optimizing management.

OUTPUTS FOR PRACTIONIONERS

G. lutea is neither uniformly distributed nor endangered, thus regional assessments are crucial to identify populations of priority for conservation. Demographic and molecular studies are fragmented so a homogeneous global overview is missing (Cogoni et al., 2018). The same for its reproductive biology. The historical trend is missing and probably it would highlight a higher IUCN category even at global scale. *G. lutea* is likely suffering from climate-change, especially in their PIPPs, thus based on the above-mentioned studies, ENM can be a valuable tool. Another direct pressure is the overharvesting, thus promoting local sustainable use/cultivation would be useful.

Q1. Are there competitive weeds that cultivated gentians cannot withstand competing?

Yes: Weed control such as mowing and tree cutting is required repetedly in the first year of cultivation every 4–6 weeks and the cultivation period should span from four to six years to achieve economically viable root yields.
No: Q2

Q2. Are there trees on the top floor creating a shading canopy?

Yes: The trees should be pulled out so the canopies don't block the sunlight and create cool shadows, as well as because of the water and nutrient competition.
No: Q3

Q3. Are populations of *G.lutea* stable?

Yes: Q4
No: There should be strategies and activities implemented for reinforcing the populations, such as habitat protection, monitoring, propagation and cultivation, reintroduction, genetic diversity conservation, etc.

Q4. Are the mesurements for seed germination applied?

Yes: Q5
No: First the soils should be well provided with organic matter and potassium, with ranging from medium to strong acid pH levels, than the seeds should be treated with cold stratification for two months at 2 °C in order to overcome the seed dormancy or with gibberellic acid to enhance dormancy release and promote seed germination under a wide range of temperatures.

Q5. Is the population of *G.lutea* big and how does it affect the proportion of seeds per plant?

Yes: Species in bigger populations produce more seeds, approximately 8000 seeds per plant, in spite of that, the seed size decreased with the expansion of the population size. Nevertheless, total seed mass increased with the size of population. With potential ex situ conservation, seeds should be collected only from larger populations due to seed germination success in 57.5% cases in some studies.
No: Species in smaller populations create fewer seeds, around 4000 seeds per plant, nonetheless, individual seeds that are produced are larger that those species that are in bigger populations.

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GUIDELINES

for species conservation

General guidelines for *Gentianella preaecox ssp. bohemica* conservation

Kateřina Iberl

Gentianella preaecox ssp. bohemica

General guidelines for *Gentianella preaecox ssp. bohemica* conservation

Kateřina Iberl

Introduction

Since the industrial revolution the number of extinct and endangered species threatened species has increased drastically (Dahl, H.-J., Niekisch, M., Riedl, U., Scherfose, V.). The Red Lists published annually by the IUCN (International Union for Conservation of Nature and Natural Resources) illustrate this for the last few decades and point out the future risks (Bates et al., 2024).
For this reason, all states and regions are requested international conventions, in particular Convention on Biological Diversity (CBD, Rio de Janeiro 1992) to conserve diversity (CBD, 2022).

Gentianella bohemica, the Bohemian gentian, is a species that only occurs in Austria, the Czech Republic and the border region of Germany.

The taxon *Gentianella praecox ssp. bohemica* is listed in Annex II and IV of the European Union Habitat Directive 92/43/CEE of 21 May 1992 “Animal and plant species of community interest whose conservation requires the designation of pecial areas of conservation” (Council of the European Community 2007); however, without explicit mentioning its different flowering morphs which are often taxonomically treated as subspecies. Mainly for that reason, we use the epithet *bohemica*, here, although the valid name is G. praecox (A. et J. Kerner) Dostal ex E.Mayer, while G. bohemica Skalicky in its strict sense only refers to the late-flowering morph [syn. G. praecox (A. et J. Kerner) Dostal ex E.Mayer subsp. *bohemica* (Skalicky) Holub], whereas the early-flowering morph was described as G. gabretae [syn. G. praecox (A. et J. Kerner) Dostal ex E.Mayer subsp. praecox (Skalicky) Holub].

The species is critically endangered according to the Red Lists of Czech Republic, Germany, and Austria.

G. bohemica is an endemic to the Bohemian massif and a Czech subendemic. Its historic distribution area includes the Czech Republic (most of the territory except W and NW Bohemia and SE and E Moravia), north Austria, the W part of Lower Bavaria and southernmost Poland.

In the past, the taxon occurred throughout most of the area of the Czech Republic, in the mesophytic and oreophytic belt (rarely in the thermophyticum). It grew especially in pastures of the association Cynosurion, low-grass meadows of the associations Violion caninae and Nardion, in some mown mesic and moister meadows of the associations Arrhenatherion and Molinion, and in many disturbed habitats (e.g., road verges, edges of quarries and wood margins).

The decline in the number and extent of localities of the species is probably related mainly to changes in land use (cessation of grazing, especially by sheep and goat, eutrophication, succession processes, deliberate afforestation etc.). During the last approximately five years, extreme weather conditions also seem to play an important deleterious role, particularly in dry types of habitats.

Without suitable management measures, populations will only survive in the soil seed bank. However, estimates of he seed bank characteristics suggest only a limited seed viability period restricted to approximately seven to ten years. During this time slot, the viability of buried seeds was proved to reduce rapidly.

In Czech Republic, a research regarding the distribution, population biology and ecology of *G. praecox ssp. bohemica* took place in the years 1999-2001. Moreover, data for the rescue program were collected for the Ministry of Environment within a particular study in 2003. Later on, further research continued under the program “Priorities related to the species conservation of vascular plants” which was running from 2006 to 2011. The rescue program for the species was elaborated in 2010.

In Bavaria, long-term attention is given to research on *G. praecox ssp. bohemica* and to the deployment of measures at its localities (coordinated by the Bayerisches Landesamt für Umwelt). Similar action plans are starting up in Austria (coordinated by the Österreichische Naturschutzjugend Haslach). In Poland, targeted protection of the species is at its beginning.

SCIENTIFIC PART

1. General information about the species

1.1. Taxonomy

Gentianella praecox (A. & J. KERNER) E. MAYER ssp. *bohemica* (SKALICKÝ) HOLUB was described in 1969 (Skalický V., 1969) under the specific name *Gentianella bohemica* SKALICKÝ, which appeared to differ from previously described taxa *G. germanica* (WILLD.) BÖRNER and *G. austriaca* (A. & J. KERN.) HOLUB. The European taxa of the genus *Gentianella* have been basically divided into three morphologically differing groups: first, *G. amarella* agg., second, *G. campestris* agg., and third, *G. germanica* agg. (Greimler et al., 2004). The first two groups are mainly coherent, whereas the third one is more heterogeneous. The study species *G. bohemica* belongs to the highly diverse last group (Skalický V., 1969; Wisskirchen, Rolf, Haeupler, Henning, 1998) and is described as an intermediate taxon between *G. germanica* s.str. and *G. austriaca* from the geographical and morphological viewpoint (Skalický V., 1969; Greimler et al., 2004; Jang et al., 2005). The taxonomic treatment of the early and late flowering morphs differs at the species level (Skalický V., 1969), at the subspecies level (Holub, 1998), and even under the subspecies level (Greimler et al., 2004).

1.1.1. Nomenclature

G. bohemica is listed as a priority species on Annexes II and IV of the Habitats Directive (Council of the European Union, 2007). However, in this document there is no explicit mentioning its different flowering morphs which are often taxonomically treated as subspecies. Mainly for that reason, we use the taxonomic name *Gentianella bohemica*, although the valid name is *Gentianella praecox* (A. et J. Kerner) Dostal ex E.Mayer. In a strict sense, *G. bohemica* Skalicky refers solely to the late flowering morph [syn. *G. praecox* (A. et J.Kerner) Dostal ex E.Mayer subsp. *bohemica* (Skalicky) Holub], whereas the early flowering morph was described as *Gentianella gabretae* [syn. *G. praecox* (A. et J. Kerner) Dostal ex E.Mayer subsp. *praecox* (Skalicky) Holub] (Plenk et al., 2016).

1.1.2. Variability

Similarly to many other taxa, the genus *Gentianella* forms two seasonal flowering morphs (Wettstein, 1900; Skalický V., 1969; Fischer R., Oswald K., Adler W., 2008). The two flowering morphs of *G. bohemica* show different morphological characteristics in their adult stage: the early flowering morph is sparsely branched, has long internodes and often only a few flowers. In contrast, specimens of the late flowering morph are usually well branched, have short internodes and numerous flowers. Branching in the lower part of the stem can, however, be a result of mechanical disturbance caused by mowing or grazing (Plenk et al., 2016 according to Gotz 1991, pers.comm.). Flowering time of the early flowering morph is early summer (usually second half of June), whereas the late flowering morph flowers in autumn (in September and October). Their seeds ripen during the late summer to late autumn, repsectively. The early morph occurs in the Lower Austria, whereas the late morph occurs exclusively in Czech Republic, Germany and Poland.

Is predominant in Austria and Within the same population, there can be great variability in reproductive traits, e.g. the number of flowering stems per plant can range between

1.1.3. Karyology

The Chromosome number is (2n): 36, the ploidy level (x): 4.

The 2C genome size [Mbp] is 3889.69, 1Cx monoploid genome size [Mbp]: 972.42
The Genomic GC content corresponds to 39.7 %.

1.1.4. Hybridization

Czech Republic: following hybrids are known (Kirschner and Kirschnerova, 2000): *G. obtusifolia* ssp. *sturmiana* × *G. praecox* ssp. *bohemica*, *G. germanica* ssp. *germanica* × G. praecox ssp. bohemica, G. × macrocalyx (ČELAK.) DOSTÁL (= G. campestris × G. praecox ssp. bohemica), *G.* × *austroamarella* MORAVEC ET VOLLRATH (= *G. amarella* ssp. *amarella* × *G. praecox* ssp. *bohemica*). Currently, only the hybrid *G.* × *austroamarella* is perceiving in the species distribution area (locality Opolenec). Here, the taxon has been described by Moravec and Vollrath (1967). This taxon poses no threat to nature conservation.

1.2. Species distribution and conservation status

1.2.1. Species area

G. praecox ssp. *bohemica* is an endemic of the Bohemian Massive. Its historical distribution area comprises Czech Republic, northern Austria, Bavaria and southernmost part of Poland. It once occurred on almost the entire territory of the Czech Republic except for west and north-west Bohemia and south-east and east Moravia. It has been credibly recorded from over 650 localities in Czech Republic and a few dozen historical localities in Austria, Poland and Bavaria. Since the year 2000, it is known from only 111 localities despite intensive searching. The recent population number (2023) in the Czech Republic is 77 (out of this number, only 48 have been "vital localities" (i.e. with at least one flowering individual during the last ten years). Further, thirty localities are known in Austria (… of which are vital localities), eight in Germany (six of which are vital localities during the last ten years), 4 in Poland (2 of which vital localities).

1.3. Biology and ecology

1.3.1. Phenology

Flowering in August/September/October (the early morph in April), fruiting in September/October/November, (early morph in …), germinating in spring.

1.3.2. Life form and strategy

Gentianella bohemica is a strictly biennial species. Germinated seeds develop to seedlings and rosettes during the first year. The 1-year-old plants form rosettes carrying flowering stems in summer (the early morph) or in autumn, respectively (the late morph).

S-stress-tolerator.

1.3.3. Reproduction

1.3.3.1. Generative

- Reproductive system: mixed mating system
- The proportion of flowering plants, the number of flowering stems and flowerheads produced per plant and seed set increase with population size
- Age at first flowering: 2 years
- Soil seed bank: persistent
- Dispersal strategy: mainly anemochory

1.3.3.2. Vegetative

Does not occur.

1.3.4. Plant life cycle

The species is strictly biennial. Seedlings germinate in spring and the plant grows. One-year-old plants form rosettes and overwinter. Two-years-old plants produce flowering stems which are capable of flowering. Plants produce seeds in autumn of the second year. Seeds either give rise to seedlings the next spring, or they build the seed bank.

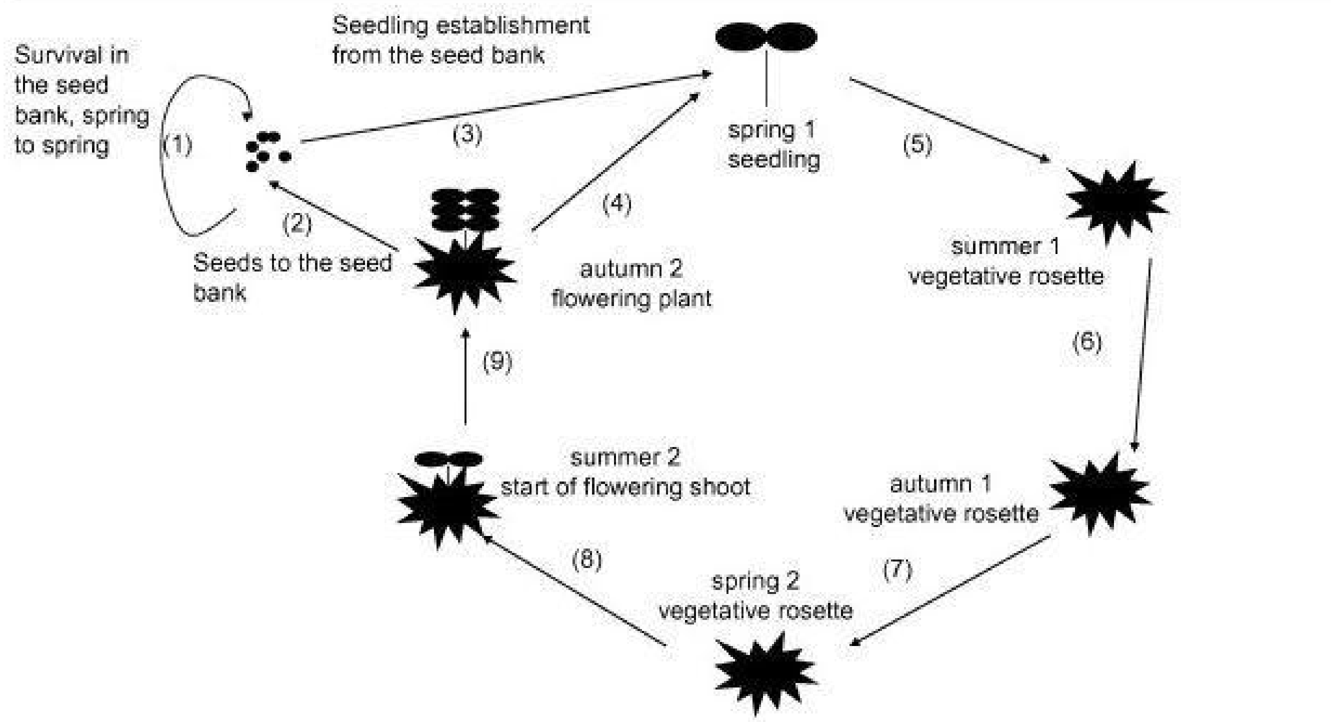


Fig. 1. Life cycle of G. praecox ssp. bohemica. Bucharová et al. 2012

1.3.5. Habitat

Abiotic conditions

Chemical analyses of the soils displayed a wide range of abiotic conditions at the thirteen monitored sites. At a depth of ca. 5 cm the soil reaction varied from acidic (pH 4.7) to slightly basic (pH 7.7), which was correlated with the contents of Ca (661–7898 mg.kg-1) and Mg (52– 1204 mg.kg-1) ions. The sites were poor to moderately rich in nutrients. Total carbon content varied from 0.9 to 11.9%, nitrogen from 0.1 to 0.8%, exchangeable phosphorus from 2.8 to 19.3 mg.kg-1.

Ellenberg ecological indicator values: L: 8; T: 5; M:4; R: 5, N: 3

Phytosociological alliances

Bromion erecti (broad spectrum of grassland types classified as Subatlantic broad-leaved dry grasslands), *Arrhenatherion elatioris* (extensive hay meadows), *Violion caninae* (species-rich Nardus grasslands), *Koelerio-Phleion phleoidis* (dry grasslands on acidic soils).

EUNIS habitat type: R1A32 (Bromion), ….

1.3.6. Biotic factors

1.3.6.1. Herbivory

Often attacked by slugs, especially one-year old rosettes. Spring is presumably the most vulnerable time, when the seedlings and rosettes emerge.

1.3.6.2. Pollination

Pollinated predominantly by Hymenoptera (bees, bumblebees). Diptera (Syrphidae)? Lepidoptera?

1.3.6.3. Symbiosis

Association with arbuscular mycorrhizal fungi

1.3.6.4. Antagonism

Diseases:

1.4. Species threats

1.4.1. Current

Lack of disturbance.

Mowing adjusted to leave comparatively tall stands. This practice was often recommended to avoid destruction of young plants and rosettes. Alternatively, extensive grazing was promoted. However, when restoring, and/or maintaining sites, it is essential to create gaps through disturbance. Thus, cutting as low as possible combined with raking-up and proper removing the biomass combined with scarifying is essential. Alternatively, intensive rotational grazing is advisable. The aim is to disturb the turf and to create small gaps before germination starts. This takes place approximately at the turn of April and May, depending on the altitude, grassland type, etc.

Bad timing

The management must not be carried out at the time of growth, flowering and seed ripening of the gentians, i.e. roughly from end of June to mid-October (late morph). Conversely, intensive farming (mowing twice a year, rotational grazing) from mid-October to the end of June in the following year is ideal.

Absence of seed bank activation

Although management in spring partly leads to disturbance of plant development (cutting of branches followed by compensational branching) and to direct destruction of rosette seedlings (up to 30% of present rosettes), at the same time it lowers competition and enables germination from the short-term or long-term seedbank. This approach compensates for the losses by up to tenfold. As demonstrated in experimental studies (Brabec et al. 2011, Bucharová et al.2012), germination from the seedbank is the most important factor in the life cycle of this biennial species – and at the same time the one best to be influenced by farming.

1.4.2. Future

Environmental conditions become less predictable due to the climate change. Especially dry conditions in spring seem to be unfavorable for young rosettes, as the juvenile plants are sensitive to drought. Possible solutions: To compensate for current and future stressful abiotic conditions less suited to the ecological requirements of the species. In the current context of climate change, the species seems to have found refuge in the moving shadow, as well as due to a specific microhabitat relief (empirical observations on certain sites).

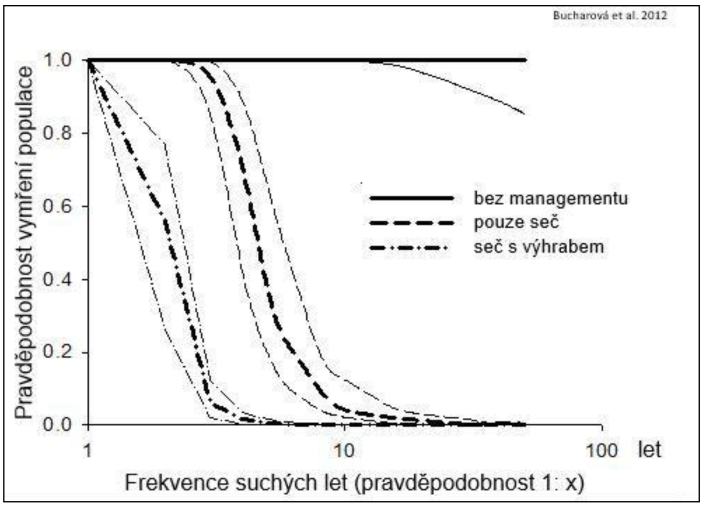


Fig. 2. Extinction probability of a small population (10 flowering individuals) as a function of extremely dry years. 95% confidential intervals are indicated by thin lines. Full line: no management; dashed line: mowing only; dashed & dotted line: mowing and disturbance. Bucharova et al. 2012

1.5. Previous implemented management interventions

1.5.1. In-situ

In situ conservation actions were undertaken in Czech Republic, Germany, Austria and Poland. Particularly, clearcutting, stems removal, mowing, raking and harrowing. As an integral part of the site management, scarifying has been implemented (Czech Republic). For more detailed description, see the chapter 3.

1.5.2. Ex-situ

Seed banking

G. praecox ssp. *bohemica* has desiccation-tolerant seeds, i.e. the seeds can be stored in dry-cold conditions while maintaining their viability for germination.

In the years 2004 and 2005, seeds of *G. praecox* ssp. *bohemica* were collected from the site Protivanov, Nature Reserve U Žlíbku and stored in the Seed Bank of endangered plant species, based in the museum "Vlastivedne Muzeum" in the city of Olomouc, Czech Republic. Viability control of the long-term stored seeds was not carried out.

Multiplication

In case there was a sufficient amount of seeds, usually it was also possible to cultivate rosettes and flowering individuals. However, in most cases only a limited number of individuals could be raised. It seemed that plants grown in experimental conditions were more susceptible to disease. This might be due to the absence of natural vegetation cover and thus enhanced draught, but possibly also due to the absence of an appropriate mycorrhiza. Thus, cultivation seems to be a challenging task.

Overall production of mature seeds by cultivated plants usually did not exceed the number of seeds used for cultivation.

We are not aware of any successful case of long-term cultivation maintained exclusively by harvested seeds.

In view of the fact that the metapopulation dynamic is missing (i.e. missing of natural colonization process from surrounding populations), new populations can only be established if a sufficient seed bank is built. Currently, this is only possible if seeding takes place repeatedly, using high amounts of seeds (thousands rather than hundreds) from a source population. At the same time, optimal management is necessary. To conclude, starting a new population of *G. praecox* ssp. *bohemica* is a long-winded process requiring constant efforts. It would only make sense if several large populations occurred in the vicinity. This would enable the seed translocation for several subsequent years from the source(s) to the target site. In any case, the necessary prerequisite is a well-maintained site providing the best possible conditions required by the species. Further, a proper management must be guaranteed for several years in advance.

2. Aims of rescue plans

2.1. Selection of priority populations

2.1.1. According to the criteria of viability

1. A population has reached at least three times a number of 100 flowering individuals during the last ten years,
2. In case of a suboptimal, insufficient, or inadequate management, a population has given a positive response to the measures. If a proper management was carried out during two subsequent years, a population recovered within two or three years since the proper management was introduced. This means the number of individuals increased sharply, and a population increased as least threefold.
3. A population has not been maintained the last five times during the last 10 years. Alternatively, the population was managed unsuitably, and despite this fact, at least three times within the last 10 years, at least 15 flowering individuals have emerged.

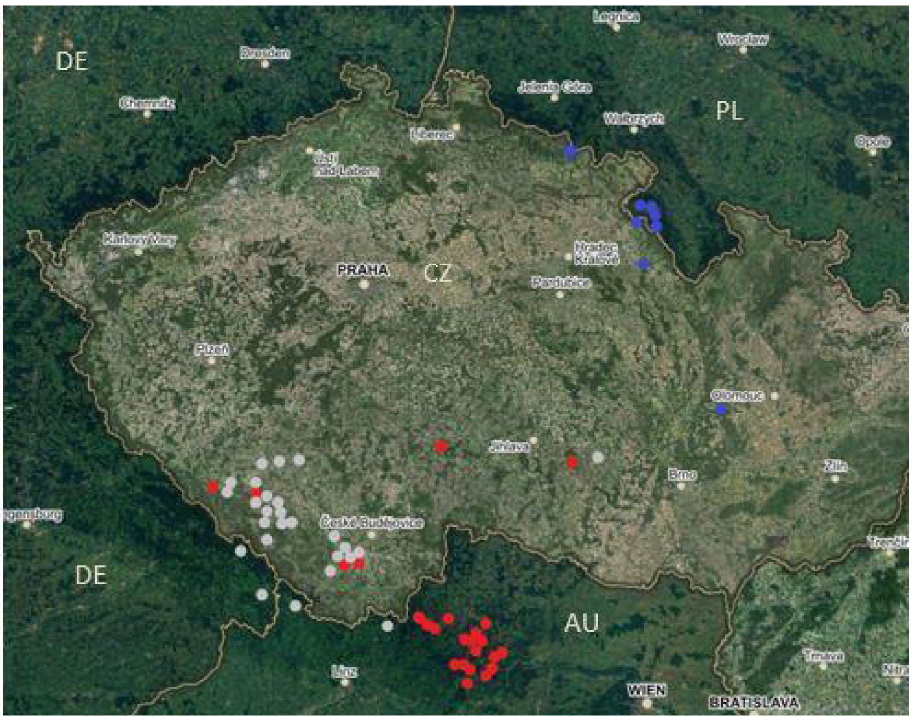
2.1.2. According to the genetic criteria

The aim is to protect the whole genetic variability comprised in surviving populations in the whole distribution area of the species. Therefore, the most genetically valuable populations, maintaining high levels of genetic diversity *and* accumulating private alleles, should be protected as priority populations (particularly, if also responding to the previous criteria of viability).

The whole distribution area consists of three distinct genetic clusters:

1. the northern cluster "The Giant Mts." comprises populations of the Giant Mts., the Orlicke Mts. (Czechia), the Góry Stolowe (Poland) and its surroundings.
2. The south-western cluster "Bohemian Forest" includes predominantly populations of the Bohemian and the Bavarian Forest and its northern surroundings.
3. The south-eastern cluster "Austria" involves populations from both Lower and Upper Austria.

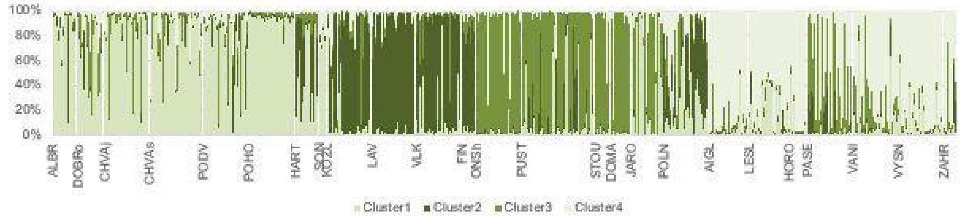
This genetic structure should be respected and should be taken into account when carrying out restoration/translocation measures.



In each genetic cluster, we have chosen a subset of populations comprising the highest levels of genetic diversity. Additionally, all populations containing private alleles were included in the population subset. Thus, we aim to recommend which populations should be prioritized in the decision-making procedures related to the practical nature conservation.

Figure... Results of the STRUCTURE cluster analysis. The 57 studied populations across the whole distribution range of the species were assigned to three genetic clusters, corresponding to the grey, red and blue dots on the map (Iberl et al., unpublished data)

Genetic group „Bohemian Forest“



Subcluster	Abbreviation	Location	He	Private Allele
1	JARO	Jaroškov	0.6393	0
1	CHVA.J	Chvalšiny JZ	0.6142	0
1	ALBR	Albrechtice	0.6240	0
1	DOBRo	Dobročkov	0.6314	0
1	CHVA.S	Chvalšiny S	0.6370	0
2	VLK	Vlkonice	0.6208	0
2	POLN	Polná	0.6355	1
2	KOZL	Kozlov	0.6603	0
3	ONS.H	Onšovice	0.5812	0
3	PUST	Pustiny	0.6091	0
4	HORO	Čistá Hora	0.3020	1
4	AIGL	Grünwald	0.4787	1
4	LESL	Mairsprindt	0.5211	1
4	VANI	Sudslavice	0.5750	1

A subset of genetically most valuable populations from the genetic group „Šumava“ (i.e. populations comprising the highest levels of genetic diversity *and/or* containing private alleles)

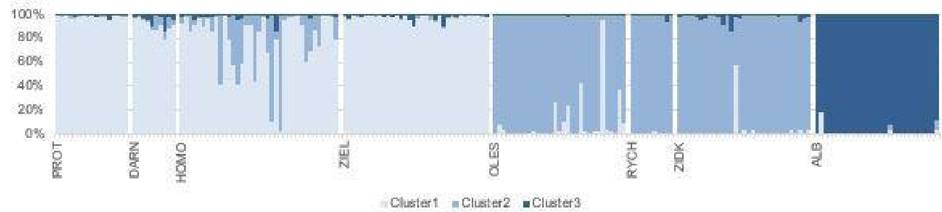
Fig. 4. Priority populations from the genetic cluster “Bohemian Forest”, containing the highest levels of genetic diversity and private alleles.

Genetic group „Austria“

Subcluster	Abbreviation	Location	He	Private Allele	Current state
1	ERNL	Ernst	0.5759	0	
1	HROBY	Hroby	0.6674	0	good
2	HOHEL	Slučí Tah	0.4253	1	very good
2	MITL	Mitterschlag	0.5153	0	
2	VOIL	Voitsau	0.5241	0	
2	GRML	Groß Meinharts	0.5312	0	very good
2	TRBL	Troibetsberg	0.5392	0	good

Fig. 5. Priority populations from the genetic cluster “Austria”, containing the highest levels of genetic diversity and private alleles.

Genetic group „Giant Mts“



Subcluster	Population	Location	He	Private Allele
1	ZIELENIEC	Torfowisko pod Z.	0.5228	0
1	PROT	Protivanov	0.5585	0
1	OLES	Olešnice	0.6203	2
1	HOMOLE	Gomola	0.6269	3
2	ZIDKOVI	Zelinkův Mlýn	0.4381	0
2	DARNKOW	Darnków	0.5119	0
2	RYCH_15	Rychnov	0.5890	0
3	ALB	H. Albeřice	0.3846	2

A subset of genetically most valuable populations from the genetic group „Giant Mts.“ (i.e. populations comprising the highest levels of genetic diversity *and/or* containing private alleles)

Mid-term aims

Medium-term objectives: For the first ten years after the action plan is adopted, the following objectives are set: 1) To ensure or optimize management at all priority localities of *G. praecox ssp. bohemica*. At each locality, it is necessary to secure high quality management of a sufficiently large area, 2) To continue with regular monitoring of all recent localities of the species, 3) To gain new knowledge about the biology and ecology of the species, 4) To ensure that stakeholders farming at localities of *G. praecox ssp. bohemica*, are sufficiently aware of the species' protection. 5) To compose a list of potential source and target populations for repatriation of the species.

Long-term aims

Long-term objectives: 1) To stop the decline in the number and size of populations of the species at at least 27 priority localities of the species in SW Bohemia, SE Bohemia, the Drahanská vrchovina region and the Tábor region. 2) To improve the state of the species' populations at five priority localities in the Českomoravská vrchovina region.

3. Suggestion of interventions

Constant farming measures are necessary to maintain the sites in a good condition. This means, not only mowing but also raking of litter and mosses during the early spring or late autumn, in order to create good conditions for germination.
The full commitment is essential: if the litter or moss layer exceeds the depth of just 1 cm, seedlings/roots of seedling would not be able to penetrate this layer and dry up.

It is favourable to maintain the localities as large as possible – even parts in which the species has never occured before(!) It has a very limited perspective, to preserve only small patches with the current occurrence of the species.

Look for diversified (micro)climate conditions on site – e.g. walking shadow of surrounding trees, specific microhabitats enabling the population to „escape" changed unfavorable conditions etc.

Never skip / omit the management measures – not a single year.

3.1. Site management

Basic measures comprise:

(1) removal of biomass (i.e., end of spring, beginning of summer – not later than middle of June (late morph, and/or in autumn October/November, after plants have matured and released their seeds). Frequency depends on the habitat type (dry / mesic grasslands) – for details see the decision tree.

(2) turf disruption, creation of gaps (harrowing, vertical cut/scarifying, or rotational grazing) for seed germination in the vegetation after maturation and release of seeds, i.e., approximately from October/November to spring, not later than middle of April

The basic approach should be modified according to the requirements of a particular vegetation type, the state and position of a locality, the number of flowering individuals and the current course of the weather during the season.

3.1.1. Tree cutting

Large-scale clearcutting measures at various sites. The cleanup including cutting of most shrubs and trees, stumps removal; complete cleaning of the site.

3.1.2. Mowing

Mowing decreases competition, improves light availability at ground level and prevents litter accumulation. Germination and seedling establishment will be higher where the vegetation is cut as short as possible short when mowing. For the late morph, yearly mowing in May/June, as well as October/November is recommended. For the early morph, cutting regime must be adjusted so that plants would not be cut during the period of flowering and ripening of seeds (.....).

Fig. 7. The patch prior mowing: a layer of living and dead biomass fully covers the soil surface. Here, no recruitment can take place. Photograph by courtesy of Jiri Brabec.

3.1.3. Cleaning / raking

After mowing, complete and very thorough raking / leaning of the site are necessary. Only if this is fulfilled, litter and biomass accumulation can be avoided. However, this measure alone still does not create the necessary conditions for germination. Mowing and raking alone are far from sufficient to maintain a population in the long term. This is because the roots of the seedlings are too weak to penetrate the moss layer. Thus, seedlings may dry out and die.

Fig. 8. The patch was maintained by mowing only. Living biomass was removed, but litter and moss layer would still hamper germination and seedling recruitment. Photograph by courtesy of Jiri Brabec.

3.1.4. Scarifying / vertical cut

To create gaps suitable for germination, but especially in order to activate the species´ seed bank beneath the soil surface, the method of vertical cut/scarifying proved to sufficiently fulfill this aim. This is a promising technique of turf disturbance. It is vital to supplement mowing and raking with this method, as the germination from the seed bank is a crucial phase in the life cycle of the species.

Fig. 9. The patch was maintained by mowing, raking and scarifying. The site is well prepared for germination and seedling establishment. Photograph by courtesy of Jiri Brabec.

3.1.5. Grazing

Grazing is a management method which was traditionally used in the sites where species occurred. This management method causes disturbance in the soil surface and thus facilitates seedling establishment. However, this does not hold in case if the pasture regime is only extensive.

3.2. Species targeted interventions

3.2.1. Ex-situ conservation

3.2.1.1. Seed banks

G. bohemica seeds maintain their viability for decades in dry-cold rooms. Seed collections in remaining populations are therefore recommended in order to capture as much of the genetic variability of the species as possible across its distribution area.

Storage conditions are those recommended by the FAO for long-term conservation: drying at 15% RH, followed by freezing at -20°C in airtight containers. Glass jars, flame-sealed vials and heat-sealed trilaminate aluminum bags represent the only truly airtight options. Any other container should be avoided.

3.2.1.2. Plant cultivation as a seed source for reintroduction

Potting soil

Recommended pH: the soil reaction in natural sites varies from acidic (pH 4.7) to slightly basic (pH 7.7)

3.2.2. Plant translocation

Sowing is the only (re)introduction possibility in this species, due to its biennial habit. However, the practice so far has shown that only immense numbers of seeds are required during several subsequent years. Thus, only full and concentrated commitment, combined with the choice of an appropriate site and an abundant seed source population(s) in the surroundings (to preserve the local genetic pattern) might bring at least small prospects of a long-term success.

		4 500 seeds		4 500 seeds	600 seeds		15 000 seeds		40 500 seeds			
2000	2003 to 2012	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
1	0	1	0	10	0	0	23	1	89	45	67	131

An example of the conservation translocation from Southern Bohemia:

4. Public awareness

The management measures on localities in Czech Republic were organized by five different Nature conservation organizations. Particularly when starting regular management or optimizing management, commitment of volunteers was very important. Their work included additional turf disturbance, but in several cases, volunteers carried out the whole cleanup on their own and managed the site (with consent of the landowner) for three years.

OUTPUTS FOR PRACTIONIONERS

Tab. ##: Causes of endangerment, their consequences and the most important countermeasures

Cause of endangerment	Consequences	Measures
Shadowing due to high trees and / or dense shrubs	Populations overgrown by trees and shrubs disappear aboveground and continue to survive in the soil seed bank (ssb) only. However, longevity of the ssb is limited to approx. 7 to 10 years, in extreme cases up to 12 years.	Removal of single trees and shrubs, if it makes sense, removal of tree trunks. In south exposed slopes, some trees should be left due to the shadow. Fast-growing shrubs and trees should be completely removed where possible. Regular aftercare and removal of competing species is essential (Guidelines Chapter 3.1.1)
High Competition through herbs and mosses	Inactivation of the soil seed bank. Impaired seed germination; decay of young rosettes due to competition	Yearly management not only through mowing, but also, essentially, through removal of mosses and old dead biomass by scarifying and raking. Thus, competition of tall herbs and grasses is reduced and ssb activated (Guidelines chapter 3.1.2., 3.1.3., 3.1.4.)
Insufficient management practices	Lack of activation of the ssb, dead biomass and competing mosses /grasses hamper seed germination and development of rosettes	Technical discussions with responsible land owners, NGOs and authorities who carry out management. Purchase and management of valuable sites by nature conservation authorities / foundations or rental and management by nature conservation professionals.
Inappropriate timing of mowing	Destruction of flowering stems and buds (too early summer cut before flowering (during shooting); loss of seeds removed together with biomass in unopened capsules (too early autumn cut, before immature seeds were released from still closed capsules)	Adherence to the recommended mowing times (Guidelines Chapter 3.1.2). Or, harvest the unripe capsules before the autumn mowing, dry/ripen and spread again in site after the mowing. This is particularly advisable in locations where there is little time between natural seed ripening and the first snow. Using this method, the ssb can be greatly strengthened and not weakened by the mowing and removal of partially still green seed capsules.

Cause of endangerment	Consequences	Measures
Small and isolated populations	High risk of population extinction. Lower overall seed production -> gradual depletion of the soil seed bank. Increasing role of potentially catastrophic, stochastic weather and population processes.	Time is running against the maintenance of the population! Immediate use of disturbances (scarifying) in March / the first half of April to activate the remaining soil seed bank. Simultaneous optimization of the mowing management (Guidelines Chapter 3.1.2 and 3.1.4)
Overgrazing	Destruction of the plants	Reducing of grazing, fencing out the population (Guidelines Chapter 3.1.5).
Damage caused by wild animals	Weakening or destruction of the plants	Fencing out the population or single plants
Damage caused by snails	Destruction of plants	In this a challenging problem, individual solutions should be found according to local conditions
Extreme heat /draught in south-facing locations	No rejuvenation due to extremely difficult establishment of seedlings	Avoid complete clearcutting of trees and shrubs. Extend mowing/scarifying management also to new parts of sites with specific protecting microrelief etc.
Climate change	Unpredictable weather conditions hampering rejuvenation due to reduced flowering; low survival rates of seedlings and rosettes due to prolonged drought periods	Storing seeds in seedbanks (Guidelines Chapter 3.2.4). Searching new possibilities within current locations (water sources, microrelief, “walking” shadow). Moreover, searching for appropriate sites for reintroduction efforts using seeds – e.g., higher elevations with more humid and cooler climate, slopes exposed preferably to east or west). Ex situ – cultivation if possible, sowing seed in new locations

Decission tree

The decision tree key can only be used as a basic orientation tool. Depending on the vegetation type, condition of the site, location of the site, a number of flowering individuals of *G. bohemica* and a current weather during the season, the basic procedure can be variously modified.

1. Locality overgrown with trees and/or shrubs ....2.
- locality free of trees and/or shrubs..5.

2. Clearcutting. South-facing (or south-west, south-east) inclination of the location..3.
- North-facing (or north-west, north-east, west, east) inclination of the location..4.

3. It makes sense to leave a few trees standing because of the shade. Removal of stumps. The stumps should be partly or completely pulled out if it makes sense; complete mowing and cleaning of a site, removal of litter by raking, turf disturbance by harrowing, levelling with light machines (avoid destroying existing micro-relief on the site!), or vertical cut / scarifying.

4. Removal of trees/shrubs. Removal of stumps. The stumps should be partly or completely pulled out if it makes sense; complete mowing and cleaning of a site, removal of litter by raking, turf disturbance by harrowing, levelling with light machines (preserve existing microrelief of the site!), or vertical cut / scarifying.

5. Cleared and / or cultivated sites in which the late morph occurs (flowering August / September (October)..6.
- Cleared and / or cultivated sites in which the early morph occurs (flowering June / July)... 13.
- Cleared and / or cultivated sites in which both the early and late morph occur..16.

6. The habitat type is a dry grassland (associations Bromion erecti / Suboceanic broad-leaved semi-dry grasslands, Koelerio-Phleion phleoidis / acidophilous dry grasslands) ....7.
- The habitat type is a mesic grassland (*Arrhenatherion elatioris* / Lowland to submontane mesic meadows, *Nardion strictae* / Siliceous alpine and boreal grasslands, *Violion caninae* / Submontane and montane Nardus grasslands)**...10.**

7. Dry grassland site with uniform site conditions...**8.**
- Dry grassland site with diversified site conditions regarding relief, inclination, compass direction, water sources, walking shadow of trees etc...**9.**

8. After the clearcutting, mowing regime twice a year is recommended, during the period of one to three years after clearcutting. Mowing to take place in May/June and in October/November. Raking up/removal of litter to follow the mowing. Every year turf disturbance by harrowing, or by performing a vertical cut since the end of October / early November, but *preferably* in early spring (not later than mid-April). After the period of one to three years after the clearcutting, mowing regime only once a year. If rotational grazing is available, then use the same timing as for mowing.

9. After the clearcutting, mowing regime twice a year is recommended, during the period of one to three years after clearcutting. Mowing to take place in May/June *and* in October/November. Raking up/removal of litter to follow the mowing. Every year turf disturbance by harrowing, or by performing a vertical cut since the end of October till the end of November or, *preferably*, in early spring (not later than mid-April). Moreover, in the face of the climate change, it is also important to *include* and to *maintain* specific (micro)sites within the localities where the species have not grown in the past (e.g. depressions in the relief, partially shaded areas etc.) After the period of one to three years after the clearcutting, mowing regime only once a year. If rotational grazing is available, then use the same timing as for mowing.

10.The habitat type is a mesic grassland in lowlands...**11.**
- The habitat type is a mesic grassland in mountains...**12**.

11. The first mowing May–June, the second mowing October/November, when flowering is complete and seeds are ripe, capsules open; this can vary from year to year. Biomass removal by means of raking up the litter; turf disturbance by harrowing or by performing a vertical cut/scarifying after the cut, approximately since the end of October, in November or, preferably, in early spring (not later than mid-April). If grazing is available, then use the same timing as for mowing.

12. First cut May to June, not later than flowering start of *Arnica montana*, second cut from mid-October/November, when flowering is complete and seeds are ripe, capsules open; this can vary from year to year. Scarifying in autumn since the end of October, during November (depending on the snow cover), but preferably not until spring to protect the site against possible black frost (not later than mid-April / depending on the snow cover). If grazing is available, then use the same timing as for mowing.

13. Early morph in dry grasslands...**14.**
- Early morph in mesic grasslands..**15.**

14. Dry grasslands in which the early morph occurs should be cut once a year. Mowing only after the seeds have matured and been released from the capsules, approx. from August to October. Raking should follow after the mowing. Turf disruption / scarifying from mid-October to the end of November (mid-December). If rotational grazing is available, then use the same timing as for mowing.

15. Mesic grasslands in which the early morph occurs should be cut once or twice a year, (depending on specific site conditions and / or weather course each year), not before the end of flowering, after the seeds have ripened and detached from the capsules, approximately from August to October. Raking should follow after each mowing. Turf disruption / scarifying from mid-October to the end of November (mid-December). If rotational grazing is available, then use the same timing as for mowing.

16. The occurrence of both morphs (early and late) in one location is a rare case, so that an individual decision on management is necessary.

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