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The relationship between climatic conditions and generative reproduction of a lowland population of *Pulsatilla vernalis*: the last breath of a relict plant or a fluctuating cycle of regeneration?

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Abstract The alpine-boreal plants which have survived in Central European lowlands during glacial periods depend both on the preservation of their refugial habitat, and their capability for vegetative and generative reproduction and dispersion. *Pulsatilla vernalis* (L.) Mill. is a model species which occurs throughout most of the European alpine system, as well as in isolated populations in the lowlands. At present, the relict lowland localities of this species often have a historic character. In the Polish lowlands, only the population located in Rogowiec is characterized by effective recruitment. It presents a large number of rosettes and a diversified demographic structure, with plants in all stages of development. The study examines the population in Rogowiec from 2002 to 2008 with regard to the number of flowering and fruiting shoots, new seedlings, and loss of juvenile rosettes, and the obtained data are correlated with climatic conditions. Three years were found to demonstrate effective recruitment, with numerous seedlings and little loss of juvenile rosettes. No significant relationship was found between seedling dynamics and the flowering–fruiting process. However, correlations were found between effective renewal and some climatic factors. Temperature, water balance, and solar radiation were found to have a limiting effect on the reproduction and regeneration of the analyzed relict population. Due to the longevity of the rosettes, favorable climatic conditions occurring every few years are sufficient for survival of the species in this location. However, serious threats are posed by the climate change trends expected in Europe over the coming decades, and the fact that due to its small-scale dispersal ability, the analyzed species occupies only a small area in Rogowiec.

Keywords Alpine-boreal plant · Relict species · Flowering · Fruiting · Germination · Seedling survival · Effective recruitment · Climatic conditions

Introduction

The distribution of plant species is influenced not only by current environmental conditions, but also the history of vegetation, both of which have been subjected to continual change in the Quaternary. These have resulted primarily from fluctuations in climate between glacial and interglacial stages (Willis and Niklas 2004). The ability to survive in refuges can enable some species to survive unfavorable climatic periods and to widen their ranges following beneficial changes of climate (Hampe and Jump 2011). Relict

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populations allow us to gain a better understanding of the processes shaping the geographical distribution of plants (Jackson et al. 2009). Such localities can, on the one hand, be a remembrance of earlier ranges and afford an insight into the history of the species, and on the other, act as source-populations from which the taxon may re-expand in the case of future climate change. Populations evolving in isolation from others can also be a very valuable source of genetic diversity (Reisch et al. 2003).

Increasing molecular evidence indicates that some arctic-alpine plants have survived in the Central European lowlands during glacial periods in so-called cryptic refugia (Bennet and Provan 2008; Stewart et al. 2010; Michl et al. 2010). Their enduring existence at lowland sites depends on refugial habitat preservation, as well as their capability for vegetative and generative reproduction and dispersion (Birks and Willis 2008; Schmitt et al. 2010). Many cold-adapted plants from the alpine belt present a stress-tolerant life strategy (S), which is characterized by longevity, small size, long ontogenetic development, and low vegetative growth (Grime 1979; Crawford 2008). Moreover, in a lowland forest landscape, the wind dispersal vector typical of open arctic or alpine habitats is ineffective. As a consequence, the existence of alpine-boreal plants in lowlands depends on in situ reproduction. Recent years have seen many populations of such species destroyed by human activity. In addition to those which were directly destroyed, there are populations which disappear despite the apparent presence of a suitable habitat for them. Although the reasons for their disappearance reported in the literature are often the results of loose observations not supported by in-depth research, there is a limited but rapidly growing body of empirical evidence which suggests that the current climate constrains the reproduction and regeneration of relict populations (Hampe and Jump 2011).

The generative process in an exceptionally well-preserved population of glacial relict *Pulsatilla vernalis* (L.) Mill. in the Polish lowlands was examined with respect to climatic conditions in permanent plots over a seven-year period. At present, the relict lowland localities of *P. vernalis* in Central Europe and in the south of Fennoscandia are threatened by extinction or have a high threat status (Grzyl and Ronikier 2011 and cited references, Fig. 1). Nowadays, most of them have a historic character and are represented only by herbarium records from the turn of the 20th century. In the last decade, only 26 of over 200 historically-noted populations were observed in the Polish lowlands (Grzyl and Ronikier 2011). More than 60 % of the remaining populations have fewer than ten individuals and only two populations number over 200 plants. A similar situation is observed in another countries: for example, in Sweden, more than 60 % of populations have fewer than six plants (Åström and Stridh 2003).

The lowland *P. vernalis* population in Rogowiec, Central Poland, is noteworthy in that it stands in contrast to the other, decaying, populations (Hereźniak et al. 2001). The large number of rosettes, and the diversified demographic structure with plants in all development stages demonstrating the population dynamics, cannot be observed in any other lowland locality in Poland (Grzyl and Ronikier 2011). This population offers the opportunity to carry out studies based on the flowering–fruiting process, seedling germination, and viability of the species, which can be unique for this part of its geographical range. Because the existence of alpine-boreal plants in the lowlands depends on in situ reproduction, and *P. vernalis* reproduces exclusively in a generative way, studies concerning the initial phases of individual development are important both for understanding the processes that govern plant survival in the relict localities, and for the benefit of the species recovery program. Hence, the population in Rogowiec was chosen as a subject to answer the following questions:

1) What is the relationship between seedling dynamics and the flowering–fruiting process in a population?
2) Which climatic factors drive the flowering–fruiting process, germination, and seedling survival in a population?

Materials and methods

Species description

Pulsatilla vernalis (L.) Mill. is a model species which occurs throughout most of the European alpine system, including the subalpine and alpine habitats of the Pyrenees, the Alps, the Carpathians, and the Balkans, as well as the Scandinavian chain (Fig. 1). The species also occurs in the isolated relict

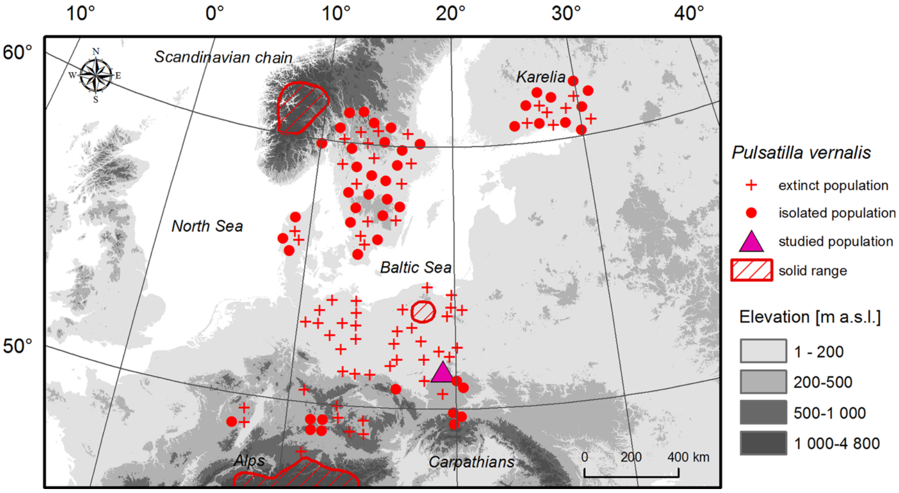

Fig. 1 Localization of studied population of *Pulsatilla vernalis* on with respect to its occurrence in Central Europe and Southern Fennoscandia (according to Ronikier et al. 2008; Grzyl and Ronikier 2011, changed)

population in the German, Polish, Norwegian, Danish, Swedish, and Karelian lowlands (Meusel et al. 1965; Ozenda 1985; Muller 1997; Ronikier et al. 2008, Fig. 1). Phylogeographical studies of *P. vernalis* suggest the presence of a higher diversity of haplotypes in mountain areas compared with those in lowland populations, and supports the opinion that *P. vernalis* originated in alpine habitats and subsequently colonized the lowland areas during glacial climate changes (Ronikier et al. 2008). The genetic data have also shown that the presence of *P. vernalis* in the lowlands of Central Europe is not the result of a recent (Holocene) post-glacial colonization from the Alpine refugia. Rather, it indicates an earlier migration from mountain to lowland areas and survival in northern periglacial refugia, located between the Alps in the south and the Scandinavian ice sheet in the north (Ronikier et al. 2008). During the early Holocene, the lowland populations from territories in what is now Poland and Germany may well have been the sources of further northward migration to Scandinavia (Ronikier et al. 2008).

Pulsatilla vernalis survived in the lowlands in pine forests (*Dicrano-Pinion*, *Vaccinio-Picceetea*) and heathlands (*Nardo-Callunetea*). In Poland, some extant populations also occur in anthropogenic habitats such as the edges of clearings, roadsides, and the slopes of railway embankments inside forest complexes (Grzyl and Ronikier 2011). The decline of populations is correlated with the natural succession of long-term forest from pine to mixed tree-stands, accelerated by the anthropogenic eutrophication of soils.

The individuals of *P. vernalis* pass through several stages: juvenile, immature, virginile, generative, and senile, each characterized by different morphological features (Simačev 1978). In the case of juvenile, immature, and virginile plants, the individual produces a single rosette, but individuals in the generative stage can produce several. It is worth noting that the only form of reproduction of this species is generative, all rosettes belonging to a single individual constitute a unity and cannot function separately. One generative rosette can produce between one and three flowering shoots (Simačev 1978). *P. vernalis* is a long-living perennial, and each developmental stage can take a different number of years, for example, in the population in Rogowiec, individuals were observed to pass through juvenile stages lasting from three to more than 8 years (Grzyl 2012). There are no tests to determine the maximum age of a rosette, but it is known that their ontogenetic development preceding the generative phase can reach more than 10 years (Simačev 1978).

Population of *P. vernalis* in Rogowiec

The study was conducted in the vicinity of Rogowiec village situated near Bełchatów in Central Poland. This is one of the two locations of *Pulsatilla vernalis* in central Poland, where more than 200 specimens grow (Grzyl et al. 2013). Until 2012, the population in Rogowiec was the only known population with a well-developed demographic structure in the lowlands. The other locations in Poland either have a very small number of individuals or lack rosettes at different ages and reproduction stages. Hence, the population in Rogowiec was the only one allowing a long-term study to be conducted. A body of evidence attests to the continuing presence of the taxon in this place for at least 30 years (Hereźniak et al. 2001). The phytocoenosis with *Pulsatilla* is located at the base of a dune hill overgrown by a private pine forest, extensively exploited by selective logging, with the trees reaching ages of about 70 years. The ground is, typically, developed podsols on loose sands containing small quantities of a clay fraction. These soils are dry and well aerated, with high porosity and a low capillary capacity to lift water (Grzyl et al. 2013).

Field research

The area inhabited by the population of *P. vernalis* was covered by a grid of 1 m squares. The delineated research area covered 500 m^2. The squares were divided into smaller squares of 0.1 × 0.1 m to enable the accurate inventory of rosettes. The counting of individuals is not straightforward, as particular plants produce several ground rosettes that share a common root system and it is difficult to know which rosettes belong to which individual. In each research plot, the number of rosettes was counted and, thanks to the morphological differentiation of the leaves, they were classified into different stages of development. Thus, information about the structure of the population was obtained.

In each year from 2001 to 2008, the population in Rogowiec was repeatedly monitored throughout the whole growing season (a few times a month) and the numbers of juvenile, immature, virginile, generative and senile rosettes were counted. In addition to counting the specimens belonging to each of these developmental categories, the number of new seedlings was noted. However, only the results obtained for the years 2002–2008 were taken into account for the analysis, as in the first year, 2001, it was difficult to clearly differentiate newly-germinated individuals from the other juvenile rosettes. The distinction of juvenile rosettes from the others was possible due to the unique morphology of their leaves. The parameters of the analysis comprised the number of new seedlings, the total number of juveniles and their mortality. The number of generative rosettes and the total number of rosettes were also incorporated into the analysis. Also, the number of flowering shoots produced by generative plants was counted, as was the number of fruiting shoots: the latter being lower as a result of such causes as grazing by forest animals.

Climatic data

Climatic data were taken from the meteorological station located 1 km from the *P. vernalis* population in Rogowiec (IMGW Annual Reports 2001–2008). The original data include the following monthly climatic parameters: (a) temperatures (°C): mean diurnal—Td, minimum—Tmin, maximum—Tmax; (b) moisture: vapor pressure—VP (hPa), relative humidity—RH (%), saturation deficit—SD (hPa); (c) insolation—I (h); (d) precipitation—P (mm).

The studied period was standard for climatic conditions in central Poland (IMGW Annual Reports 2001–2008). Maximum precipitation took place in the summer months, and low precipitation was noted during the winter periods. During the seven studied years (2002–2008), the mean diurnal temperature in the Rogowiec meteorological station was 9.3 °C, which was higher than the average values for the previous 50 years in central Poland, and the mean annual sum of precipitation amounted to 553 mm, which was typical for central Poland.

Data analysis

The correlation between the population parameters of *P. vernalis* and the climatic conditions over the period 2002–2008 was evaluated using the Spearman's rank correlation coefficient. The STATISTICA PL. ver. 10 (Stat-Soft Inc. 2011) software package was used for all statistical calculations (Emden 2008). The relationship was first identified between the population parameters and climatic data collected separately for all months. As individual correlations were characterized by a high randomness, the analysis presented in the article only includes selected parts of the year, which are particularly important in terms of the *P. vernalis* phenology observed in the Rogowiec area (Fig. 2). By using the information contained in Fig. 2 regarding the process of flowering, the conditions in the months of March–April were assumed to exert a key influence on the process of fruiting in April–May, the dissemination mostly being observed in June. New seedlings

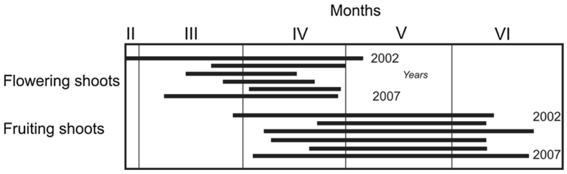

Fig. 2 General phenology scheme of the *Pulsatilla vernalis* population in Rogowiec (Central Poland) based on long-term (2002–2007) observations (according to Grzyl 2012)

Table 1 Seven-year dynamics of the flowering/fruiting process in the lowland population of *Pulsatilla vernalis* in Rogowiec (Central Poland)

Year	Flowering shoots		Fruiting shoots		Generative rosettes	Total rosettes
	n	%	*n*	%	*n*	*n*
2002	34	61.82	25	45.45	55	156
2003	15	23.08	13	20.00	65	146
2004	19	27.14	7	10.00	70	151
2005	26	31.33	19	22.89	83	158
2006	12	12.50	5	5.21	96	206
2007	50	48.08	42	40.38	104	207
2008	40	34.78	12	10.43	115	222

n Number, % percentage of generative rosettes

Table 2 Seven-year dynamics of the recruitment process in the lowland population of *Pulsatilla vernalis* in Rogowiec (Central Poland)

Year	New seedlings		Juvenile rosettes		Loss of juvenile rosettes	
	n	%	*n*	%	*n*	%
2002	**19**	**12.18**	**57**	**36.54**	**4**	**2.56**
2003	4	2.74	33	22.60	22	15.07
2004	4	2.65	26	17.22	4	2.65
2005	5	3.16	22	13.92	6	3.80
2006	**48**	**23.30**	**63**	**30.58**	**6**	**2.91**
2007	12	5.80	54	26.09	16	7.73
2008	**21**	**9.46**	**64**	**28.83**	**9**	**4.05**

Bold years have the most effective recruitment

n Number; % percentage of total rosettes

were observed in late summer (mostly in August); the conditions prevailing in this month were considered crucial for the germination of seeds. The outbreaks of new seedlings in the spring, from seeds that had survived the winter, were very sporadic (Grzyl 2012).

Results

During the seven-year observation period, the number of total and generative rosettes of *P. vernalis* in Rogowiec increased from 155 to 222 and from 55 to 115, respectively (Table 1). The highest increase of the total number of rosettes occurred in 2006 as a result of the abundant germination that year (Table 2). Changes in the numbers of flowering and fruiting shoots exhibited fluctuation dynamics (Table 1). Three seasons appeared to be the most effective for the recruitment process: 2002, 2006, and 2008. During these seasons, a high number of new seedlings occurred, together with little loss of juvenile rosettes (Table 2).

A highly significant correlation (0.89) was found between the total number of rosettes and generative rosettes in the studied population of *P. vernalis* (Table 3). A positive, but not statistically significant correlation (0.71) between fruiting and flowering shoots contributed to the effectiveness of this generative process (Table 3). On the other hand, the number of new seedlings was not dependent on the number of flowering and fruiting shoots; the correlation coefficients being −0.13 and −0.18, respectively (Table 3).

The analysis of the correlations revealed significant associations between climatic conditions and the chosen population parameters of *Pulsatilla* (Table 4; Fig. 3). A positive, but statistically insignificant correlation (0.75) was found between the number of flowering shoots and mean diurnal temperature and minimum temperature in the March–April period (Table 4; Fig. 3a). Fructification of *P. vernalis* is positively correlated to the maximum temperature (0.82), vapor pressure (0.82) and diurnal temperature (0.78) in the April–May period (Table 4; Fig. 3b). The number of seedlings and juvenile rosettes was found to have a very strong positive correlation (0.90 and 0.86, respectively) with precipitation in August (Table 4; Fig. 3c), and a negative (−0.72 with significance at $p < 0.07$), correlation with insolation during this month (Table 4).

Discussion

Our results add further support to the existing ecological research which emphasizes the limiting effects of temperature, water balance, and solar radiation on the reproduction and regeneration of relict populations

Table 3 The rank Spearman correlation coefficients between *Pulsatilla vernalis* population parameters during the 7 years observation period in Rogowiec (Central Poland)

	New seedlings	Juvenile rosettes	*p*	Total rosettes	*p*	Generative rosettes	*p*	Flowering shoots	*p*	Fruiting shoots	*p*
New seedlings	1.00	**0.85**	*0.016*	0.74	*0.058*	0.47	*0.268*	−0.13	*0.787*	−0.18	*0.699*
Juvenile rosettes				0.61	*0.148*	0.43	*0.337*	0.18	*0.702*	−0.21	*0.644*
Total rosettes						**0.89**	*0.007*	0.57	*0.180*	0.07	*0.879*
Generative rosettes								0.39	*0.383*	−0.14	*0.760*
Flowering shoots										0.71	*0.071*
Fruiting shoots										1.00	

Bold values are significant at $p < 0.05$

Table 4 The rank Spearman correlation coefficients between climatic conditions in crucial periods for the phenology of *P. vernalis* and certain parameters influencing its population

March–April	Fl	*p*	April–May	Fr	*p*	August	S	*p*	J	*p*	LJ	*p*
T max. $_{\mathrm{III–IV}}$	0.28	*0.534*	T max. $_{\mathrm{IV–V}}$	**0.82**	*0.023*	T max. $_{\mathrm{VIII}}$	−0.49	*0.268*	−0.14	*0.760*	−0.09	*0.846*
T min. $_{\mathrm{III–IV}}$	0.75	*0.052*	T min. $_{\mathrm{IV–V}}$	−0.09	*0.848*	T min. $_{\mathrm{VIII}}$	−0.34	*0.448*	0.02	*0.969*	−0.19	*0.679*
T d. $_{\mathrm{III–IV}}$	0.75	*0.052*	T d. $_{\mathrm{IV–V}}$	**0.78**	*0.039*	T d. $_{\mathrm{VIII}}$	−0.34	*0.448*	0.05	*0.908*	−0.13	*0.784*
VP $_{\mathrm{III–IV}}$	0.61	*0.143*	VP $_{\mathrm{IV–V}}$	**0.82**	*0.023*	VP $_{\mathrm{VIII}}$	0.11	*0.816*	−0.05	*0.908*	−0.60	*0.149*
RH $_{\mathrm{III–IV}}$	−0.27	*0.558*	RH $_{\mathrm{IV–V}}$	−0.41	*0.355*	RH $_{\mathrm{VIII}}$	0.70	*0.086*	0.29	*0.531*	−0.04	*0.922*
SD $_{\mathrm{III–IV}}$	0.32	*0.482*	SD $_{\mathrm{IV–V}}$	0.61	*0.148*	SD $_{\mathrm{VIII}}$	−0.67	*0.102*	−0.21	*0.644*	0.07	*0.877*
I $_{\mathrm{III–IV}}$	0.29	*0.534*	I $_{\mathrm{IV–V}}$	0.71	*0.071*	I $_{\mathrm{VIII}}$	−0.72	*0.068*	−0.43	*0.337*	0.05	*0.907*
P $_{\mathrm{III–IV}}$	−0.32	*0.482*	P $_{\mathrm{IV–V}}$	−0.64	*0.119*	P $_{\mathrm{VIII}}$	**0.90**	*0.006*	**0.86**	*0.014*	0.00	*1.000*

The data are based on the 7 years (2002–2008) monitoring of the permanent plots in Rogowiec (Central Poland). Bold values are significant at $p < 0.05$

Population parameters: *Fl* number of flowering shoots, *Fr* number of fruiting shoots, *S* number of new seedlings, *J* number of juvenile rosettes, *LJ* loss of juvenile rosettes

Climatic conditions: Analyzed periods: $_{III–VI}$ March–June, $_{IV–V}$ April–May, *T max.* maximum temperature, *T min.* minimum temperature, *T d.* mean diurnal temperature, *VP* vapor pressure, *RH* relative humidity, *SD* saturation deficit, *I* insolation, *P* precipitation

(Hampe and Jump 2011). During this seven-year study of a relict population of *P. vernalis,* regular increases and decreases in the number of flowering and fruiting shoots were observed, which followed a fluctuating dynamic pattern. The flowering–fruiting process was found to be positively related to mean diurnal temperatures during the March–May periods. In addition, the degree of fructification was found to depend on the maximum temperature and occurrence of rather sunny and dry weather in April and May. However, the water balance (vapor pressure) also was seen to have a significant influence. It is probable that the humidity in spring, after the winter snow melting, is sufficient for the growth of *P. vernalis,* and that sunny weather is needed for effective flowering and pollinator visitation (Jüngers and Dötterl 2004).

An interesting result is that the number of seedlings was not related to the number of flowering and fruiting shoots during the season. The greatest degree of germination occurred in 2006, when the number of flowering and fruiting shoots was the lowest. The seedling outbreak theoretically could be related to the seed deposit of the previous year, but seeds of this species do not require vernalization and outbreaks of new seedlings in the spring are very sporadic (Grzyl 2012). Assuming that germination observed in summer is associated with fruiting in a given year, it can be concluded that the fruiting population is more dependent on external conditions than on the presence of a large number of fruits. This is confirmed by Grzyl (2012), who studied the germination potential of seeds collected from the population in Rogowiec: under

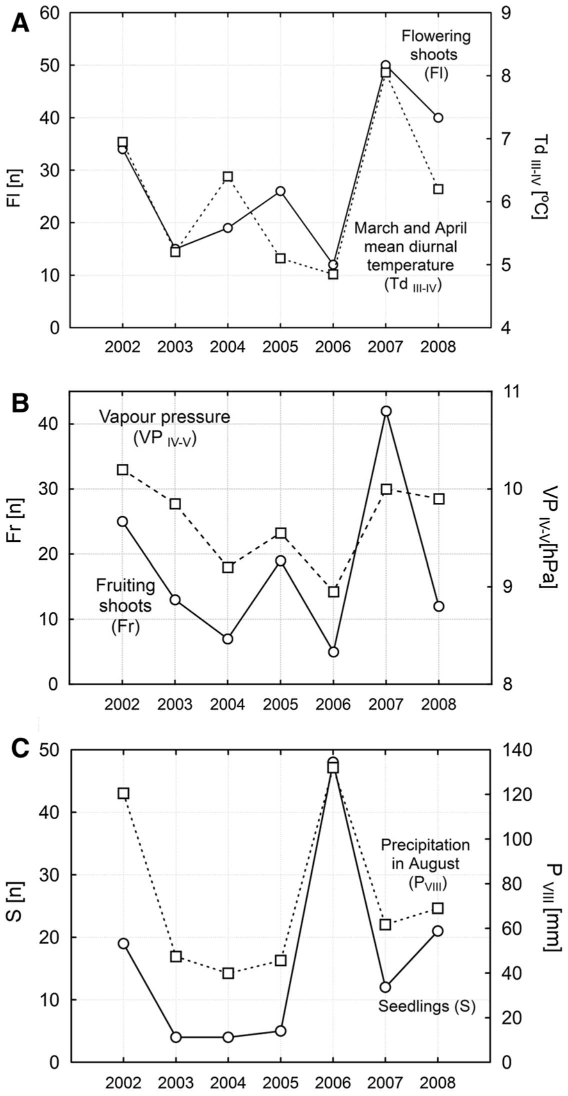

Fig. 3 Seven-year changes of flowering shoot **a**, fruiting shoot **b**, and seedling **c** number in the population of *Pulsatilla vulgaris* in Rogowiec (Central Poland) and the strongest correlated climatic conditions in the Rogowiec Climatic Station

favorable conditions, more than 90 % of the seeds germinated, and the number of wintering seeds was negligible. For reproductive success, therefore, a small number of seeds is sufficient.

Three years with effective recruitment were identified, with numerous seedlings and little loss of juvenile rosettes. The germination of *P. vernalis* in Rogowiec was found to strictly depend on precipitation in August. These parameters demonstrated strong correlation and similar fluctuations. Many studies of relict species reproduction were performed in regions with seasonal water shortage, and water deficit was identified as an overwhelming cause of recruit mortality (Hampe and Jump 2011). Our studies provide evidence that the reproduction of *P. vernalis,* an alpine-boreal plant, also depends on the water supply.

Perhaps the importance of the water supply results from the habitat type occupied by the analyzed species. As in the lowlands, *P. vernalis* occurs on sandy, permeable soils (Grzyl et al. 2013), high levels of precipitation were very important in maintaining the humidity of soils during the summer; this period was crucial for the germination of *Pulsatilla vernalis* in lowland populations. Hampe and Arroyo (2002) obtained a similar result for the relict population of *Frangula alnus* in a Mediterranean climate, in which the abundance of juvenile plants was positively related to precipitation in the year of their establishment. The presence of adequate water in the first year of establishment may enhance plant resistance to drought in subsequent years (Mendoza et al. 2009), and even irregular wet years may enable long-lived species to undergo some regeneration (Jackson et al. 2009; Hampe and Jump 2011). Jackson et al. 2009 discuss whether, in climate conditions of high variability, the survival and ranges of many species may depend on episodic recruitment. According to these authors, dynamics of environmental conditions requires a ratchet mechanism for occupying new areas by species with persistent, long-lived individuals and juveniles having narrower ecological niches than the adults. Bearing in mind that the life expectancy of *P. vernalis* rosettes is over a decade (Simačev 1978), the 2- to 4-year intervals between periods with effective recruitment observed in this study seem to be sufficient for species survival for a long period. Our conception is correct if the transition of seedlings to the next developmental stages does not depend on a sequence of favorable weather phenomena. In the course of our 8 years study, while negligible mortality was observed among rosettes at various stages other than juvenile, several rosettes were seen to pass on to later stages of development, and the number of generative individuals grew steadily. On the basis of these findings and the success of *P. vernalis* reinforcement in south-eastern Germany (Betz et al. 2013) it can be concluded that germination and the survival rate of seedlings have a key role in the species persistence.

Beyond the dependency of climatic factors identified in this article, the precondition for the renewal of *P. vernalis* is occurrence of sunny patches without a thick layer of moss or litter (Betz et al. 2013; Grzyl 2012). The widespread extinction of the species in central European lowlands are related to the phenomenon of forest undergrowth shading by trees and shrubs and to increase the thickness of the litter layer (Betz et al. 2013; Grzyl 2012). The decline of *P. vernalis* populations is exacerbated by intensive forestry practices, the afforesting of heathlands and forest edges and the direct destruction of plants by humans or animals (Betz et al. 2013; Grzyl and Ronikier 2011). In the process of disappearance of the taxon localities, anthropogenic pressures act synergistically with the population biology of *P. vernalis*. The plant is characterized by a long ontogenetic development preceding the generative phase and small-scale dispersal abilities: in current studies, juveniles were found only in the immediate vicinity of mature individuals (Ronikier 2005; Grzyl 2012). The majority of existing populations are small and mostly uniform in demographic structure, with juvenile plants occurring rarely, despite regular fruiting (Grzyl and Ronikier 2011).

Against this background, the increasing number of total rosettes of *P. vernalis* in Rogowiec is surprising and begs the question: which conditions at that site might be different from the conditions at the other declining or not reproducing ones? The increase of population in Rogowiec was enabled by cyclic recruitment and low loss of adult plants. As a result, the population maintains its status as the second largest in Poland (Grzyl et al. 2013), even compared with those located in the Tatra Mts. (Piękoś-Mirkowa et al. 2000). Other locations of this taxon in the lowlands have very similar characteristics in terms of soil and the type of plant community (Grzyl 2012, Grzyl et al. 2013), and from this point of view, the Rogowiec population can be considered as representative of the others. However, detailed analysis reflects some differences in the microhabitat conditions which are favorable for recruitment. First, the cover of the moss layer in Rogowiec does not exceed 20 %, while it is mostly higher than 80 % in another location in Poland (Grzyl 2012). Second, the content of carbon in the humus horizon is very low ($1.1\ \mathrm{g\ kg^{-1}}$) in comparison to other forest localities of this species in central Poland (Grzyl et al. 2013), which suggests that litter raking or fire events have probably occurred in the last decades. The importance of low needle mulch for the existence of *P. vernalis* has been confirmed experimentally by Betz et al. (2013) and Laitinen (2008). Finally, the population in Rogowiec is situated within an area receiving low levels of sporadic ash deposition from the Bełchatów Power Station, which has resulted in the neutralization of acidic conditions in the surface soil horizons (Grzyl et al. 2013). As the ash admixture in soil is important for *P. vernalis* germination (Laitinen 2008), this factor could also exert an influence on the studied population. All of these above mentioned habitat conditions have had a positive input on growth of *P. vernalis* in Rogowiec.

Our study indicates which climatic conditions are favorable for effective recruitment of *P. vernalis* in an adequate habitat. Even in quite optimal habitat conditions, the fluctuation of weather parameters have caused cycles of relict species regeneration. However, two questions arise: how long will the suitable conditions continue, and what are the threats? Three factors pose the most obvious threat: eutrophication of soil, low dispersion of species and future climate change.

The first, soil eutrophication, is most common reason for *P. vernalis* extinction in European lowlands, which occurs after cessation of traditional land use, such as litter raking and dry branch gathering.

Secondly, with regard to species dispersion, the observed germination occurred only close to the parent plants and within established patches, despite the large area of suitable habitats in the neighborhood, such as the open, sunny sites with little moss layer cover. The population is concentrated on a relatively small area and effective germination occurring during the observed period did not change this distribution pattern. Therefore, there is a high risk of the destruction of the population by random disturbances by herbivores such as roe deer (Grzyl, Kiedrzyński, personal observation), rodents such as voles (according to Laitinen 2008), or uncontrolled management of these private forests.

The third potential threat is the possible change of climatic conditions in the future. Prediction of climate change, over the period 2070–2099, suggests that lengths of “dry and hot” periods during summer will increase over most of Europe (Kundzewicz et al. 2006). In Central Poland, the summer drought period will lengthen by up to 10-15 days (Kundzewicz et al. 2006). Bearing in mind the high recruitment of *Pulsatilla vernalis* during the wetter Augusts, these climatic predictions suggest possible difficulties in the survival of the species in the future.

The persistence of *Pulsatilla vernalis* in relict lowland localities depends on cycles of recruitment induced by favorable climatic episodes and on the maintenance of habitat conditions. Therefore, a successful recovery program, as demonstrated by Betz et al. (2013), should make use of seedlings instead of seeds, and eliminate the causes of the rosettes decaying, respond to factors arising from changes in the habitat, as well as prevent the random destruction of plants.

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