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ANTHROPOGENIC SITES MAINTAIN THE LAST INDIVIDUALS DURING THE RAPID DECLINE OF THE LOWLAND REFUGIUM OF THE ALPINE-ARCTIC PLANT *PULSATILLA VERNALIS* (L.) MILL.

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Abstract

The Tuchola Forest (UNESCO MaB Biosphere Reserve) in Central European Lowlands is an area where the relict populations of the alpine-arctic plant *Pulsatilla vernalis* (L.) Mill. have survived since glacial periods. However, during the last several years rapid extinction of the species has been observed in this area. The aim of the study was the estimation of *Pulsatilla vernalis* extinction rate in the Tuchola Forest as well as the description of current refugial habitats. The results show that in approximately last 10 years (from 2004-2008 to 2015) 31% of the previously noted localities became extinct. The decrease in the species abundance (measured by the number of rosettes) affected almost all of still existing populations. The total decrease in the number of rosettes for the whole study area was 70%. In 2015, 88% rosettes grew within or at a distance smaller than ten meters from the anthropogenic habitats such as roads and slopes of railway embankments or lining them ditches. Based on the estimation of *P. vernalis* extinction rate we concluded that the analysed refugium is disappearing and if the trend will continue for the next few years, the species will probably vanish from the majority of the known localities.

Key words: Plant extinction, IUCN Red List, Relict populations, Anthropogenic habitats, The Tuchola Forest Biosphere Reserve.

Introduction

The Quaternary glacial-interglacial oscillations deeply influenced the distribution of plant species in Europe. Following climate changes, expansions and contractions of geographical ranges of temperate and cold-adapted taxa alternated repeatedly (Steward & Lister, 2001; Birks & Willis, 2008; Steward *et al.*, 2010). Therefore refugia of different species can be generally divided into glacial and interglacial ones: temperate species were confined to refugia during glacial periods and cold-adapted species during interglacials (Steward *et al.*, 2010). Based on this approach, contemporarily observed populations of some mountain and cold-adapted plants in the Europaean Lowlands can be considered as relict. These populations are survivors of the past core ranges of the species which spread over the lowlands during the glaciation periods.

A model species representing the described pattern is the spring pasqueflower *Pulsatilla vernalis* (L.) Mill. (*Ranununculaceae*) - a species listed in the IUCN Red List in the category of Least Concern (Chappuis, 2014). The main centres of the current (interglacial) occurrence of *Pulsatilla vernalis* are mountains: the Alps, Pyrenees and Scandinavian chain, where the species occurs in the alpine and subalpine habitats above the upper limit of the forest zone (Ronikier *et al.,* 2008). Outside these regions, the species was noted in the relict localities in lowlands: in the Scandinavian Peninsula, Karelia and the Central Europe (Meusel *et al.,* 1965). As a result, *Pulsatilla vernalis* has a disjunctive alpine-arctic range and lowland populations are considered to be connecting elements between the alpine and northern part of the species range (Zając & Zając, 2009, Meusel *et al.,* 1965). The phylogeographical analysis showed that there were at least two routes of migration into lowlands, having their sources in the western and eastern Alps (Ronikier *et al.,* 2008). The analysis conducted by Ronikier *et al.* (2008) points also to a possible occurrence of multiple migration events of the species from the mountains to the lowlands. During the glacial periods the species could occur quite abundantly in the areas located between the Alps and the Scandinavian ice sheet. Subsequently, northern (Scandinavian and Karelian) populations of the species are probably descended fromthose lowland populations and had, thus, become a step for further expansion (Ronikier *et al.*, 2008).

At the turn of the last century in the Central European Lowlands quite numerous localities of *Pulsatilla vernalis*were noted (Meusel *et al.*, 1965; Jalas & Suominen, 1989). Nowadays, the species grows only at isolated localities in Poland and Denmark (Ronikier *et al.*, 2008; Grzyl & Ronikier, 2011). The other localities in the region are probably extinct (Ronikier *et al.,* 2014). The largest still existing refugial area of *Pulsatilla vernalis* in the Central European Lowlands is located in The Tuchola Forest, UNESCO MaB Biosphere Reserve, in the northern Poland (Fig. 1). During the Last Glacial Maximum, the area of the Tuchola Forest was covered by the ice sheet but as the open habitats were made available by ice-retreat, the populations of cold-adapted and light-demanding species such as *P. vernalis* could spread to the north. The Tuchola Forests seemed to offer suitable conditions for the species because numerous populations dispersed through the whole region and have been survived until now (Grzyl & Ronikier, 2011). However, during the last several years, a rapid decline of the specimens has been observed in this area and nowadays The Tuchola Forest has become an interesting area to study the process of the range decline (Kiedrzyński *et al*., 2017). Formation and changes of geographical ranges under the environmental factors, especially in the Anthropocene era, raise many questions and much academic debate (e.g. Hampe & Petit, 2005; Stehlik, 2003; Pearson, 2006; Tzedakis *et al.*, 2013; Kiedrzyński

et al., 2017). At the same time the populations of cold adapted species are considered as one of the most threatened in the context of the observed and projected global warming (Hampe & Jump, 2011). In this context, the dynamic of *Pulsatillavernalis* occurrence in the Tuchola Forest is of great scientific importance.

According to Grzyl & Ronikier (2011) majority of populations of *P. vernalis* found in the Tuchola Forest occurred within or in the vicinity of anthropogenic habitats such as roadsides or railway embankments. There is a growing body of scientific research regarding threatened and endangered species found in habitats shaped by man (e.g. Nowak, 2005; Kirpluk & Bomanowska, 2008; Efimov, 2011; Rewicz *et al*., 2017). One of the reasons may be the similarity between some habitat conditions (e.g. light availability and competition) shaped by human activity and certain types of ancient/natural habitats of the species (Kiedrzyński *et al.*, 2015; Zielińska *et al*, 2016). Hence, the second aim of our work was the analysis of the occurrence of *P. vernalis* within anthropogenic habitats during the currently observed decline of the species in the region.

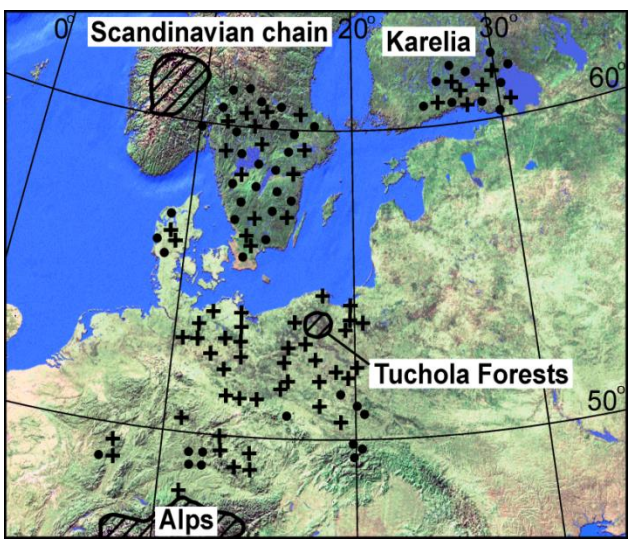

Fig. 1. Localization of the Tuchola Forest refugium with respect to the occurrence of *Pulsatilla vernalis*in Central Europe (according to Zielińska *et al*.,2016, changed); dots represent isolated localities, crosses represent extinct localities.

Material and Methods

Study area: The Tuchola Forest or the Tuchola Pinewoods is the one of the UNESCO MaB Biosphere Reserves in Poland. The area of the whole forest complex covers more than 120 000 ha, which are mostly dominated by Scotch-pine *Pinus sylvestris* stands. The terrain was formed during the last glacial period and in the periglacial conditions during the Pomeranian Phase of the Vistulian (Weichselian) glaciations, which occurred ca. 16 ka BP (Marks, 2012). As a result, most of the study area is covered by fluvioglacial sands, and is also rich in sand dunes and lakes. The main types of natural potential vegetation are sub-Atlantic pine forests *Leucobryo-Pinetum* and sub-continental oak-pine forests *Querco-Pinetum* (Matuszkiewicz, 2008).

For centuries, the area has been managed by humans; however, due to the dominance of poor soils in the region, the development of agricultural activity was restricted (Filbrandt-Czaja, 2009). Hence, the traditional exploitation of timber was the main pressure in the landscape. During the 17th and 19th centuries, human activity, eg. fires, caused the deforestation of large areas in some parts of the Tuchola Forest. Since the beginning of the 20th century, forest management has become more mechanistic and has included the planned reforestation of open sites (Filbrandt-Czaja, 2009).

Currently, in the Tuchola Forest we could find spaced groups of individuals of *P. vernalis*, which are spread across the forest complex (Grzyl & Ronikier, 2011). From the reports of foresters and local inhabitants it is known that previously the species abundance in the region was much higher, hence, historically the species could be considered as quite common there.

Study species: *Pulsatilla vernalis* (*Ranunculaceae*) is a flowering, perennial plant. The characteristic feature of that hemicryptophyte is that one rhizome can produce several rosettes, which rather cannot be separated from parent plant (Simaczew, 1978). This is why it is difficult to estimate the number of individuals in a particular locality and in most studies the number of rosettes is used as a measure of species abundance (Grzyl *et al.*, 2014).

In the mountains the taxon occurs in subalpine and alpine habitats, while in lowlands in pine forests (*Dicrano-Pinion* alliance, *Vaccinio-Picceetea* class) and in heathlands (*Nardo-Callunetea* class) (Grzyl *et al.*, 2013). There is a growing body of evidence that abundant lowland populations occur mostly in sites disturbed by periodic fires (Laitinen, 2008; Sandström *et al.*, 2017) or by human activities such as litter removal (Betz *et al.*, 2013). The species exhibits the features considered to be typical for stress-tolerant plants such as relatively low fecundity, slow growth, and an ability to grow under resource-poor conditions (Simaczew, 1978). In the landscape of lowland forests it is not able to quickly occupy the new habitats and it does not produce a long-lived soil seed bank (Grzyl, 2012).

Data collection: To estimate the dynamic changes in the number of localities and rosettes we compared the results of investigations conducted in the Tuchola Forest during the years 2004-2008 published by Grzyl & Ronikier (2011) supplemented with some more detailed unpublished data with the field research conducted in 2015. In order to objectively compare the current and previous data we adopted a precise definition of locality. As a ‘locality’ we understood a group of specimens which grows at a distance of up to 1 km from the other localities. In this way, the number of localities recorded in the Tuchola Forest area in 2004-2008 was determined at 23. All of these localities were studied in 2015. The trend and rate of *P. vernalis* extinction in the Tuchola Forest was calculated by a comparison of the number of rosettes found in the particular localities in different periods.

However, the contemporary field investigations not only included the previously known localities. During the growing seasons in 2014-2015, the Tuchola Forests were surveyed in search of the *P. vernalis* specimens according to the all available unpublished data including the information reported by Forest Services or local inhabitants. Because each locality of such endangered species is carefully noted not only by botanists but also by foresters, it can be assumed with high probability that our study included the majority of the existing localities of *P. vernalis* in the Tuchola Forests. All of the groups of rosettes found in 2014-2015 were geographically located with GPS receiver and assigned to localities according to the accepted definition of locality. The abundance of the species in each locality was measured by counting the number of rosettes.

During the field investigations we also noted the specific features of the habitats where the specimens of *P. vernalis* were found, including the location in respect to the anthropogenic structures (distance from roads or railway embankments).

Results and Discussion

During the field investigations conducted in 2014-2015 six new localities of *Pulsatilla vernalis* were found. In sum, 29 localities have ever been found in the Tuchola Forest. The total number of localities existing in 2015 was 20, which means that 31% of the localities became extinct during the last several years. The observed decrease of *Pulsatilla vernalis* localities in the Tuchola Forest was faster in comparison with related species *P. vulgaris* in England, where the decline of about 50% of localities lasted 40 years (Walker & Pinches, 2011).

In the years 2004-2008, the total number of rosettes noted in 23 localities was 450. Only in five localities the number of rosettes exceeded 50, the biggest among those was 71. Of the rosettes noted at that time, only 137 (30%) still existed in 2015, and the decrease of the rosettes number affected 21 from 23 localities. The rate of extinction was not the same in particular localities, but there was not one locality with an increase in rosettes number. When we include the new discovered localities, the total number of rosettes in 2015 was 163 and the biggest locality counted only 39 rosettes (Fig. 2). In the present study, no juvenile individuals have been observed. Such a rapid extinction rate was unexpected in the case of species which managed to survive for thousands of years in this area despite changing climate and habitats and in the case of the plants whose rhizomes could live for several decades. It was demonstrated that long-lived perennials' population declines caused by habitat destruction are likely to be delayed (Tilman *et. al.*, 1994). Then, we can suspect that conditions unfavourable for *P. vernalis* could have persisted in the Tuchola Forest for a long time (at least the last decades), and it is possible that we now observe the realization of the species extinction debt - the last stage of the species extinction in the area.

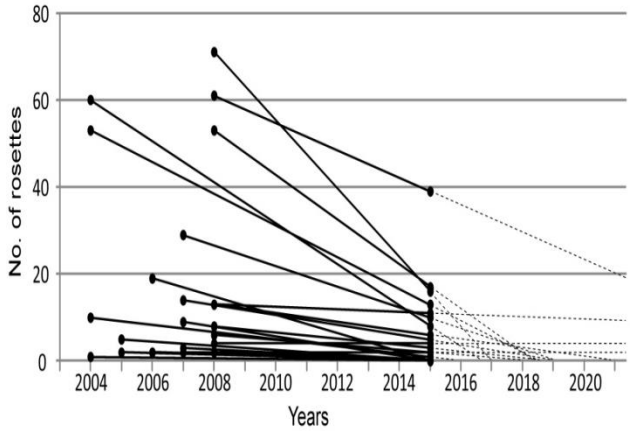

Fig. 2. Trends of decline of the number of rosettes of *Pulsatilla vernalis* in the particular localities in the Tuchola Forest determined on the basis of the previous (2004-2008; Grzyl & Ronikier, 2011) and the current research (2015; authors data).

Preservation of genetic diversity necessary for long-term existence of the population is the topic of several studies dealing with protection of endangered species (e.g. Frankham *et al.*, 2014). The attempts at determination of the minimal effective size of population (number of individuals required for proper genetic diversity) had been undertaken long ago and the number was generally estimated to be 50 individuals (Frankel, 1980; Soulé, 1980). However, several authors indicated that this number is underestimated by several orders of magnitude (Lynch & Lande, 1998; Frankham *et al.*, 2014). Even with the most optimistic approach, it can be assumed that the whole population of The Tuchola Forest has already fallen below or quickly approaches the threshold of the minimal effective population size. The additional threat comes from a very fast rate of the species decay in the region. There are authors arguing that the most pronounced effects of genetic erosion on fitness-related traits of plants occur in case of populations whose large plant abundance has declined over a short time period (within a few generations) (Ouborg & van Treuren, 1995; Hensen *et al.*, 2004). It needs to be noted that the number of individuals of *P. vernalis* growing in The Tuchola Forest, can be significantly smaller than the reported number of rosettes because a single genet can produce from one to several rosettes. Taking all this into account we may conclude that *P. vernalis* in the Tuchola Forest is on the verge of extinction and in consequence this important lowland refugium of the species in Central Europe can soon disappear.

In the case of *Pulsatilla vernalis*, light-demanding anduncompetitive species, some natural disturbances such as local fires could trigger an increase the population size (Laitinen, 2008; Sandström *et al.*, 2017). The number of individuals in a population can therefore be affected by fluctuations from one such event to another. However, the current mechanistic methods of management introduced after such disturbances allow only to a limited extent for spontaneous regeneration of vegetation. Although the human disturbances in forests can be quite similar to natural disturbances (Lindenmayer & McCarthy, 2002 and cited literature) logging can favour only the species capable of quick occupation of new areas. In case of *P. vernalis* the low opportunity of dispersion and irregular recruitment of new plants prevents the species from fast

reaching and occupying disturbed places (Grzyl *et al.*, 2014). Additionally, the process of preparing soil for the new plantings, such as plowing, can destroy existing specimens, and also the technique of dense planting of new trees results in almost complete shading of forest floor when the planted forest is young.

The aforementioned reasons probably caused the decline of *P. vernalis* from the forest interior during the last years and in the Tuchola Forest the species currently grows mostly in the vicinity of anthropogenic structures, which are associated to the long-term canopy openness. Almost all of the rosettes found in the studies conducted in 2014-2015 grew in the vicinity of linear anthropogenic structures such as roads or railways, some of them even strictly within such anthropogenic sites on the roadsides or slopes of railway embankments (Table 1). As many as 143 (88%) rosettes grew within or at a distance smaller than ten meters from the anthropogenic structure. Only two rosettes were found at the distance longer than 15 meters. Our other detailed studies of selected localities of *Pulsatilla vernalis* in the Tuchola Forest showed that the light conditions and the higher cover of the mineral soil (soil deprived from plants) together with a less competitive structure of vegetation are factors related to anthropogenic linear gaps in analysed forests (Zielińska *et al.*, 2016). Those factors can be favourable for the species occurrence but it does not mean that the presence of anthropogenical forest gaps in canopy is sufficient to the species maintenance in the study area (Zielińska *et al.*, 2016).

Because of the high scientific and conservation value of the Tuchola Forest as a lowland refugium of *P. vernalis* active protection of the species should be taken into consideration. Our study has shown that the rapid extinction of the species took place in the last years, hence the program of population reinforcement is currently needed. The first attempts were already undertaken. In 2008, experiments in seeds germination were conducted (Nawrocka-Grześkowiak & Frydel, 2012). Those experiments showed that the plants are very sensitive to pathogens during the first period of growth but the subsequent trials of planting of the obtained seedlings in the forest were completed successfully. Such a program of reinforcement of *P. vernalis* has been applied to several relict populations in low-mountain localities in southern Germany for more than ten years and with great success (Betz *et al.*, 2013). It should be noted, however, that the reinforcement of population is not enough if we do not care about the maintenance of suitable habitats. Intentional shaping of proper habitats conducive to the maintenance of the species has become a necessity. First of all, there is need for keeping open forest canopy in habitats characterised by the appropriate level of humidity and low level of interspecific competition (Zielińska *et al.*, 2016; Sandström *et al.*, 2017). The majority of the localities have been noted outside the core area of the biosphere reserve in managed forests located in its buffer zone. These are state forests so there are real opportunities for successful protection of the species provided the species conservation plan for the whole area of The Tuchola Forests will be developed. Such a plan of active protection has been developed only for Kaliska Forest District so far (Nawrocka-Grześkowiak & Frydel, 2012).

Table 1. The number of rosettes of *Pulsatilla vernalis* noted in 2015 in the Tuchola Forest classified by different habitat types (as the vicinity of an anthropogenic structure we understand the distance up to 15 meters).

Habitats	No. of rosettes in 2015
Structures accompanying railways(embankments with ditches and fire protection breaks) and the forest communities lying in the vicinity of such structures	33
Roadsides of asphalt roads and forest communities lying in the vicinity of such roads	7
Roadsides of roads paved with the crushed stone or other material and forest phytocoenoses lying in the vicinity of such roads	40
Roadsides and forest phytocoenoses lying in the vicinity of the forest service roads	83
Sites distant from any anthropogenic structures	2
Total	**163**

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