

DENSITY-BASED ASSESSMENT OF *Adonis vernalis* ABUNDANCE IN NATIVE HABITATS

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Abstract

The study investigates the ecological distribution and abundance of *Adonis vernalis* in six natural habitats, identifying significant heterogeneity in species abundance. The analysis of *Adonis vernalis* habitats is an important step in the better understanding of this species place in the community assemblage related to their native environment: Plants of *A. vernalis* were counted in 25 m² small scale plots from six specific habitats. The data was analysed towards a proposal for the potential distribution of plants in relation to a gradual habitat type.: There were identified two distribution scenarios in relation to the potential distribution of the plants in the habitat. Most of the small-scale plots showed an average of 50 plants/25 m², while in three of the habitats were identified patches with more than 200 plants/25 m². The forecast for plant presence and distribution indicates two possible outcomes - the first one indicates a maximum pick value of 30-40 plants/25 m², and the second one shows two potential picks, at 30 plants/25 m² and 120 plants/25 m². The *A. vernalis* habitats present non-linear distribution patterns across the analysed small scale-plots, with the alternation of homogeneous with heterogeneous areas. Given its medicinal value and conservation status, we propose sustainable harvesting strategies that align with the ecological characteristics of the species.

Key words: grassland, heterogeneity, habitat dissimilarity, small-scale assessment.

INTRODUCTION

The conservation of plant biodiversity is an urgent issue due to the rapid decline in the distribution of many species of wild vegetation caused by natural resource use, climate change and other factors (Zhmagul et al., 2024). Many of the endangered plants are of real interest both for maintaining biodiversity and for medicinal use or improving agricultural yields (Kardol et al., 2018; Isbell et al., 2011).

The Ranunculaceae family, distributed almost worldwide, is considered one of the essential groups of ancient angiosperms, has a history of about 75 million years according to fossil records (Anderson et al., 2005; Emadzade et al., 2010; Karahan et al., 2022).

The genus *Adonis* L. (*Ranunculaceae*), widespread in temperate areas of Asia and Europe, depending on the taxonomic concept applied includes between 20 and 40 species, frequently 32 herbaceous annual or perennial species (Aneva et al., 2019; Ghorbani et al., 2008; Karahan et al., 2022; Shang et al., 2019).

Genetic diversity is a fundamental prerequisite for species evolution and regulates the ability of populations to adapt to a range of factors such as e.g. global warming and is an important conservation target (Blows & Hoffmann, 2005; Hirsch et al., 2015).

Adonis vernalis L. forms a pontic-panonic sub-element, widespread in grasslands of the southeastern steppe zone of Europe and is characteristic for various xerothermic forests of continental-sub-Mediterranean type, belonging to the order *Festucetalia valesiaceae*. In Poland it is a relict species of the steppe zone flora (Denisow et al., 2008). *A. vernalis* is widely used in medicine in the treatment of cardiovascular diseases (Gostin, 2011; Shang et al., 2019 Zhmagul et al., 2024). As it is considered to be a threatened species, the species has been included in the IUCN Red List with the threatened status in Europe - Least Concern, although in some areas of Europe the threatened status may be assessed - extinction risk (Niculescu, 2023), it is also included in the synoptic red list for Central Europe, the species

is assessed as vulnerable by Schnittler and Gunther (1999).

The age characteristics of *A. vernalis* have been studied in different areas, resulting in the fact that the species has poor vegetative mobility, complexity of seed reproduction and low level of competition with other species in plant communities (Sultangazina et al., 2020).

Adonis vernalis is widely used in cardiovascular treatments due to its cardiotonic glycosides (Shang et al., 2019). However, overexploitation has led to a decline in wild populations, necessitating sustainable harvesting guidelines (Ghorbani et al., 2008).

The aim of this paper is to analyse the distribution and abundance of *Adonis vernalis* in 6 different natural habitats. The proposed objectives were designed to answer three different research questions: *i*) which are the limits of *A. vernalis* plant variability in their natural habitat?; *ii*) which are the potential limits of *A. vernalis* in their natural habitats?; *iii*) at what level can be considered the natural habitats similar one to each other and how the similarity varies between habitats?

MATERIALS AND METHODS

The research areas were located on 3 grasslands around the city of Blaj (Alba County, Romania). Each of these regions were selected based on their potential to offer the ecological conditions for *A. vernalis*. The grassland with plots MF1, MF2 is located SW of the city, in a Natura 2000 habitat (Mănărade Grasslands - ROSAC0428 - Semi-natural dry grasslands and scrubland facies on calcareous substrates (*Festuco-Brometalia*)). The grassland with plots OS1 and OS2 is located in the southern area of the city, and the one with samples OT1 and OT2 is in the same area, at a distance of 2 km. All grasslands areas are used through extensive grazing with cattle (in the period May-July), but without the application of any improvement management.

To accomplish the proposed objectives, 6 plots of 800 m² were selected in 2024 in the three targeted regions of study. Each plot was separated in 32 sub-plots, after an 8 x 4 scheme, that lead to a dimension of 25 m²/sub-plot (2.70 m x 8.25 m). The selection of research plots was performed based on the presence of native *A. vernalis* in canopy and the presence of cattle for

grazing. All the observation were performed in 2024, at the flowering stage of *A. vernalis*.

All data analysis was performed with the software Rstudio version 2024.12.0 (R Core Team, 2024), based on the multitude packages and statistical test. The packages used for basic statistics and histograms were “psych” (Revelle, 20024), “ggplot2” (Wickham, 2016) and “ggridges” (Wilke, 2024). Means, standard deviations and errors, medians, minimum and maximum of abundance were used to describe the abundance of *A. vernalis* in each of the six habitats. Histograms were created based on the abundance values observed in each habitat. The analysis of similarity (Anosim) was performed with „vegan” package (Oksanen et al., 2022), for the assessment of differences between and within the habitats in terms of plants abundance.

RESULTS AND DISCUSSIONS

The species *Adonis vernalis* is one of the species with conservation priorities at national and international level, it is a characteristic species for xerothermic grasslands of the continental-submediterranean type, belonging to the *Festucetalia valesiaceae* order (Zajac, 2009). Different studies (Denisow et al., 2014) state that regarding the spread of *A. vernalis* populations, they have decreased considerably in several European countries, especially in recent decades. The main causes are overexploitation of populations (gathering for medical purposes) habitat disappearance (i.e. intensive agricultural practices) or overgrowing of patches due to secondary succession of grasslands (Forycka et al., 2004; Mihalik et al., 2000; Zajac, 2009). Some causes of the disappearance of *A. vernalis* populations are also found in the biology and ecology of the plant, slow growth or problems with seed germination.

The six specific habitats of *A. vernalis* show a large difference in terms of basic statistics applied on plant number (Table 1). The means largely varied from 32 plants/plot up to a maximum of 113. Three of the analysed habitats (MF1, MF2 and OS1) showed means under 40 plants/plot, while two of the habitats showed average values of plants/plot of 63 (OT1) and more than 80 (OT2). Habitat OS2 is the only one where the mean of plants/plot reach 113 plants.

The calculated median of plants/plot in each habitat show a slightly different perspective than the calculate means. The difference observed compared to the mean for the habitats MF1, MF2 and OS1 is in the interval of 1-2 plants, with a similar situation for habitat OT2. On the opposite, for the habitat OS2 is visible an increase of median with 9 plants, while for OT1 was observed a decrease with 20 plants.

A. vernalis is a perennial plant that creates imposing tufts up to 50 cm high. The plants begin flowering in early April. *A. vernalis* is a species that reproduces generatively in natural conditions. The development of *A. vernalis* plants is slow and requires a particular composition of soil and proper weather conditions (Jankowska, 1988; Zhumagul et al., 2024a, 2024b).

The fluctuations in the comparison of means and medians are due to the minimum and maximum recorded in the field. The minimum values for MF1, OS1, OT1 and OT2 is 5 or bellow, while for OS2 is 22. These values are in contrast with the maximum recorded. Maxima values are visible in the habitat OS2 (240), to which only OT2 show close values (40 plants less). For the three habitats MF1, MF2 and OS1, the maximum values are near or below 100 plants/plot. Thus, the range obtained from ground counting is 218 for OS2, in the interval 160-200 for OT1 and OT2, and below 100 for the rest of three habitats.

The standard deviations calculated for the entire set of data indicate higher variability in each of the analyzed habitats (Table 1). For habitats MF1, OS1, OT1 and OT2, this parameter represents up to 60-80% of the recorded mean, while for OS2 less than 50%. The standard error is set in the range 5-10% for all the habitats, which enables the use of this parameter to calculate the maximum biological potential for each.

Table 1. Limits of *A. vernalis* target habitats based on the number of plants/25 m²

Plot	Mean	SD	Median	Min.	Max.	Range	SE
MF1	36.69	25.49	35.00	3.00	100.00	97.00	4.51
MF2	33.09	13.13	31.50	10.00	73.00	63.00	2.32
OS1	31.56	21.55	32.00	1.00	97.00	96.00	3.81
OS2	112.97	56.69	121.50	22.00	240.00	218.00	10.02
OT1	62.59	52.69	49.00	1.00	165.00	164.00	9.31
OT2	82.75	60.58	80.00	5.00	197.00	192.00	10.71

Legend: SD – standard deviation; SE – standard error.

According to recent literature on *A. vernalis* populations, their persistence and development in habitats of high ecological value are closely linked to the dynamics of natural phytocenoses (Denisow et al., 2014; Lohvynenko et al., 2019). Factors influencing these dynamics, such as vegetation succession, natural disturbance regime and interactions between component species, play an essential role in ensuring optimal conditions for germination, growth and reproduction of *A. vernalis* (Denisow et al., 2014; Kulymbet et al., 2023). Thus, maintaining an ecological balance in these ecosystems is essential for the long-term conservation of populations of this valuable species (Gostin, 2011; Shang et al., 2019).

Although favourable habitats for *A. vernalis* are still widespread, populations of this species are subject to significant threats, mainly due to the abandonment of phytocenoses (Ghorbani et al., 2008; Łuszczynski and Łuszczynski, 2009). The lack of traditional interventions favours the excessive expansion of shrub species and other woody plants, which leads to profound changes in the structure of the vegetation. Dry grassland with *A. vernalis* has historically been extensively grazed with sheep and maintained by burning (Onoe, 2011; Nota et al., 2020). Fire events, in particular, act to increase *A. vernalis* population (Deák et al., 2014) when happens before flowering, while the occasional spring fires stimulates the germination of this species. One of the major effects of this process is the reduction of light at ground level, an essential factor for the germination and optimal development of *A. vernalis*. As habitats become increasingly shaded and competition for resources increases, populations of the species gradually decline, risking fragmentation and even local extinction (Sultangazina et al., 2020). Understanding its distribution and ecological requirements is essential for developing conservation strategies that maintain grassland biodiversity and its possible sustainable use for pharmaceutical purposes.

The frequency of *A. vernalis* distribution (Figure 1) in the plots form the analysed habitats show a large variation, due to the growth characteristics of this species. The trends of plant number distribution across all the six habitats (Figure 1) indicates that most of the analysed plots have a maximum of 50 plants/plot. The trend slowly is

decreasing in the interval 50-150 plants/plot, followed by a reduction near to zero observations. The pick value of plants is 40/plot

and an abrupt decrease up to 75 plants/plot (first abrupt decrease), and a smooth decrease line up to the maximum of 240 plants/plot.

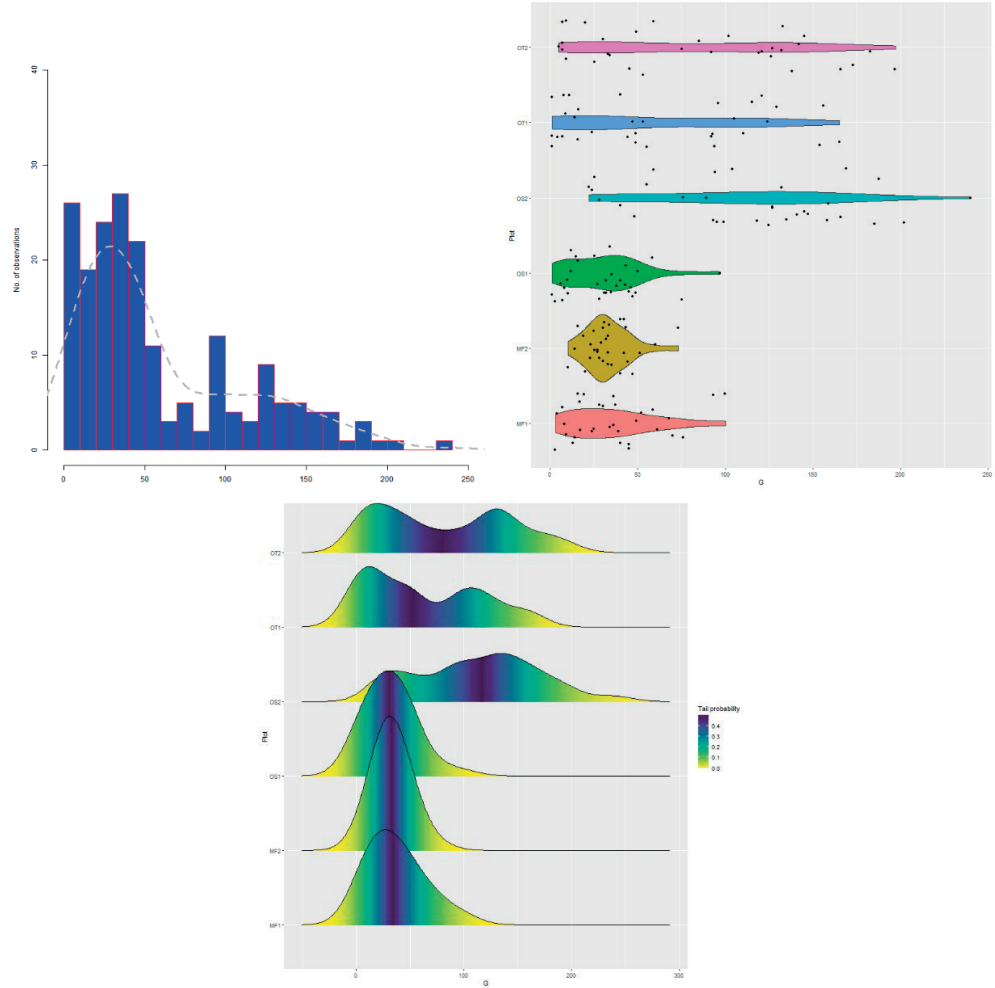


Figure 1. Distribution frequency of *A. vernalis* in natural habitats – presence vs. forecast

A case study conducted in the Akmola and North Kazakhstan regions highlights that the seed productivity of *A. vernalis* is significantly influenced by spring-specific climatic conditions, as well as by the stage of plant development (Sultangazina et al., 2020). Environmental factors, such as temperature, precipitation and the length of the growing season, play an essential role in the process of seed formation and maturation. Also, the age of the plants considerably influences the reproductive capacity, with more mature

specimens generally having a higher seed production compared to young ones. Thus, annual variations in meteorological conditions, correlated with the biological cycles of the species, determine fluctuations in the reproductive success of the species in this region (Dmytrash-Vatseba, 2016; Sultangazina et al., 2020). By analysing each of the habitat separately it can be observed a specific trend of plant distribution related to 25 m² plots (Figure 1). Three of the habitats (MF1, MF2 and OS1) show only one

probability of distribution, with a similar pick value of 30-40 plants per plot. This indicates a more homogenous distribution of plants in the field. The other three habitats show a two-tail probability curve, with one around 10 plants/plot and more than 120 plants/plot the second tail. The presence of two tails indicates a heterogenous distribution of plants in the field, with areas that present a large coverage of species and areas with sparse plants.

The number of plants at habitat level shows high fluctuations, with both heterogenous and homogenous distribution of plants (Figure 1C). For MF1, MF2 and OS1 the analysed plots show an equal distribution of observations across the entire interval of variation. For the rest of the three habitats, the distribution is across a longitudinal gradient, with a large variation between analysed plots in each habitat. These three habitats also show the high range between internal plots, with areas where plants are sparse and almost the same number of areas with a multitude of plants.

In the analysis of population dynamics of *A. vernalis*, one of the defining features is the length of the pregenerative period, which varies depending on the type of habitat. But why is this aspect so important? (Poshkurlat, 1969). First, if we look at cenopopulations in steppe areas, we observe that plants go through a much longer pregenerative period. This phenomenon can be explained by the more difficult environmental conditions: drier soil, reduced nutrient resources and large temperature variations impose on plants a survival strategy based on the slow accumulation of resources. Thus, young individuals spend several years in juvenile stages, before reaching maturity and contributing to reproduction (Denisow et al., 2014; Sultangazina et al., 2020).

In contrast, in grassland habitats, where the soil is wetter and richer in nutrients, plants no longer need a long period of vegetative development (Denisow and Wrzesień, 2006; Denisow et al., 2008). Here, the resources necessary for growth are more easily accessible, allowing for faster maturation and, implicitly, a reduction in the duration of the pregenerative stage. Consequently, populations in this environment have different dynamics, with a higher replacement rate of mature individuals. This variation in the duration of the pregenerative

period directly influences the age structure of the population, an essential factor in determining its sustainability. A balanced population must contain a sufficient number of individuals at all stages of development: seedlings, juveniles, adults and senescent plants. If the age structure is unbalanced – for example, if there are too few young individuals capable of replacing mature plants – the population may be in danger of decline. This aspect is also crucial from the perspective of the population's self-sustaining capacity. In steppes, where plants regenerate more slowly, any external intervention – such as excessive grazing or harvesting for medicinal use – can have devastating long-term effects. In grasslands the regeneration capacity is higher, allowing the population to maintain its balance more easily. Therefore, the study of age dynamics and the pregenerative period in *A. vernalis* is not just a theoretical aspect, but one with concrete implications for the conservation of the species. Understanding these mechanisms can help develop effective strategies to protect habitats and maintain stable populations over the long term.

Distribution in habitat, along with cover in plant community, is a biological characteristic of *A. vernalis*. Thus, between the 6 selected habitats is visible a large dissimilarity, determined by the number of plants present in each habitat and their allocation in sampling plots (Figure 2, Table 2). The ANOSIM analysis presents a significant difference between habitats, which is important for the detection of multiple distribution characteristics of this species.

There is a gradual, non-linear, increase in habitat dissimilarity along with the increase in the number of plots compared (Figure 2, Table 2). The lowest values of this indicator are visible when a low number of plots are compared. As the number of plots compared increase, it is visible that the increase of dissimilarity. For the entire set of plots, the dissimilarity shows high values, which is representative for a high heterogeneity of plant distribution across all the habitats. On the other hand, when the dissimilarity is compared within habitats, there are three different phenomena.

The first one is related to MF1 and MF2 habitats, where the plants are distributed heterogenous across the entire sampling area (Figure 2, Table 2). This produces a heterogenous pattern, with

the alternance of areas that present a medium number of plants with areas that present sparse plants.

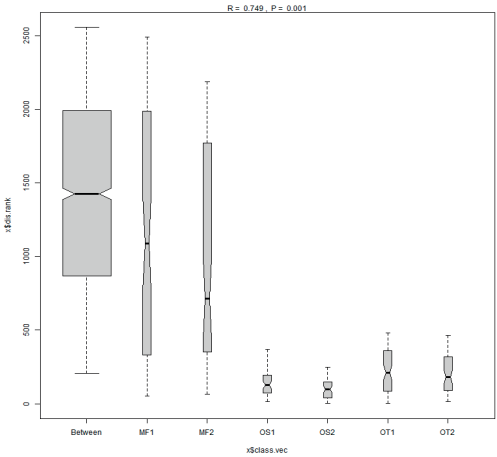


Figure 2. Similarity and dissimilarity of *A. vernalis* natural habitats

Table 2. Limits of *A. vernalis* target habitats based on the number of plants/25 m²

	0%	25%	50%	75%	100%	N
Between	206	867.75	1425.5	1990.25	2556	2160
MF1	52	343	1089.5	1968.25	2489.5	66
MF2	67	359.5	716	1747.5	2187	66
OS1	16	73.75	127.5	192.5	368	66
OS2	5	42.5	97	145.5	250	66
OT1	1	88.25	212.5	357.5	483	66
OT2	14	90	181.5	316.25	466	66

Note: ANOSIM statistic R: 0.7488 / Significance: 0.001.

The second phenomenon is visible for habitats OT1 and OT2, that present low to medium number of plants within the habitat, but their pattern of distribution is more homogenous within the habitat (Figure 2, Table 2).

The third phenomenon is composed by two distinct situations (Figure 2, Table 2). Habitat OS1, that presents medium to low number of plants, but with a homogenous distribution across the habitat. And OS2, where the number of plants is in the range medium-high, but their distribution is homogenous across the habitat, making the cross from one plot to another one smoother.

Given the medicinal importance of the species, we recommend further research on domestication potential and agro-ecological cultivation techniques. The sustainable harvesting of *Adonis vernalis* is crucial to prevent population decline, as the species has a slow regeneration

rate and is highly sensitive to habitat disturbance (Denisow et al., 2014; Sultangazina et al., 2020). Studies indicate that overharvesting, primarily for medicinal purposes, has led to significant reductions in population densities across Europe, particularly in fragmented grassland ecosystems (Forycka et al., 2004; Mihalik et al., 2000). To ensure long-term conservation, experts recommend harvesting only mature individuals after seed dispersal, limiting annual collection to no more than 30% of a local population (Ghorbani et al., 2008; Shang et al., 2019). Additionally, habitat management strategies such as rotational harvesting, controlled grazing, and protection of key populations in Natura 2000 sites are essential for maintaining ecological stability (Łuszczynski & Łuszczynska, 2009; Niculescu et al., 2023). Future research should focus on the potential for cultivating *A. vernalis* in controlled environments as an alternative to wild collection, reducing pressure on natural populations while maintaining medicinal resource availability (Gostin, 2011; Hirsch et al., 2015). Economic valorisation of grasslands through the sustainable use of medicinal species, such as *Arnica montana*, has been shown to significantly increase landowners' interest in maintaining these habitats, thereby contributing to biodiversity conservation (Păcurar et al., 2023). Studies indicate that when traditional hay meadows and oligotrophic grasslands are managed for high-value products, local stakeholders become more engaged in habitat preservation, ensuring the long-term ecological sustainability of these ecosystems. Integrating advanced monitoring techniques, such as those presented in the study "Detection and Quantification of *Arnica montana* L. Inflorescences in Grassland Ecosystems Using Convolutional Neural Networks and Drone-Based Remote Sensing" by Sângeorzan et al. (2024), can significantly enhance the accuracy and efficiency of population assessments for medicinal plant species like *Adonis vernalis*. By employing unmanned aerial vehicles (UAVs) equipped with RGB sensors and advanced image analysis algorithms, researchers can effectively detect and quantify *A. montana* inflorescences, facilitating more precise monitoring of their habitats. Applying similar methodologies to *A. vernalis* could improve

conservation efforts by providing detailed spatial data on population distributions, aiding in the development of sustainable harvesting practices and habitat management strategies (Sângeorzan et al., 2024).

CONCLUSIONS

Our findings indicate significant habitat variability in *A. vernalis* populations, with implications for both conservation and sustainable use.

The *A. vernalis* natural habitats show large heterogeneity phenomena, with a fluctuating abundance of plants even at small scale analysis. The presence and abundance of *A. vernalis* species varies greatly between different natural habitats, with an average of 50 plants/25 m², and a site-specific trend of abundance. Based on field observations the highest number of *A. vernalis* plants/25 m² can reach 240 individuals, which is considered a very abundant habitat type.

The natural abundance of *A. vernalis* shows two presence scenarios. The first one represents a homogeneous distribution of plants, with a pick value of 30-40 plants/25 m². The second scenario indicate a heterogeneous distribution, with two specific cases – 10 plants/25 m², respectively 120 plants/25 m².

High-density populations such as those in OS2 could support controlled harvesting, while low-density habitats like MF1 require conservation priority and habitat restoration.

Conservation strategies should focus on maintaining open grassland ecosystems through sustainable land management practices. Long-term monitoring is essential to evaluate the impact of ecological changes and inform adaptive conservation measures.

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