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Diversity of the Seed Material of Selected Plant Species of Naturally Valuable Grassland Habitats in Terms of the Prognosis of Introduction Success

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Abstract: The current conservation status of semi-natural grassland habitats in Poland indicates that actions for their restoration are necessary. Many of the degraded sites require the introduction of diaspores of representative species because other methods of improving their condition are not sufficiently effective. Meanwhile, little is known about the diaspores of native wild-flower species and the biology of their seeds. The aim of the present study was to find an answer to the question of which features of the seed material can guarantee the success of the introduction. The study covered 28 plant species of 4 non-forest natural habitats (codes: 6440, 6410, 6510, 6210) occurring in river valleys. Diaspores were collected in 2015–2017. Morphometric measurements of diaspores were performed, the weight of 1000 diaspores was determined and the germination capacity analysis was carried out in accordance with ISTA Rules. The analysis was made with the division into normal seedlings, abnormal seedlings, dead seeds and fresh ungerminated seeds. Species with similar parameters of seed material were selected using the method of hierarchical clustering and PCA analysis. Three groups of species were distinguished: (1) with good seed germination capacity (above 65%), which, regardless of weather conditions during the generative development, and despite the small size of diaspores, can guarantee successful introductions (*Verbascum thapsus, Veronica longifolia, Daucus carota, Plantago lanceolata*); (2) species of little suitability for introduction, due to the large proportion (over 50%) of dead seeds (*Armeria maritima, Linaria vulgaris, Potentilla erecta, Centaurea stoebe, Sanguisorba officinalis, Cnidium dubium*); (3) species with relatively large size of diaspores and low seed germination capacity, due to the high proportion of fresh ungerminated seeds which means dormant seeds (*Lathyrus pratensis, Geranium pratense*).

Keywords: grassland restoration; herbaceous species; wild-flowers; germination capacity; diaspores; seed size; seed weight

1. Introduction

The anthropogenic origin of the majority of grasslands in Poland makes the flora of meadows and pastures one of the most endangered components of the vegetation. The area of floristically valuable, semi-natural meadows in Poland, as in many European countries, is systematically decreasing [1–3]. The problem of the disappearance of some species and entire meadow communities associated with extensive forms of management is signaled by many authors [4–6]. The main cause of this phenomenon is the abandonment of the current use, which leads to uncontrolled plant succession towards communities with low natural value [7].

The current conservation status of semi-natural grassland habitats in Poland, in the continental region (monitoring, reporting period 2013–2014 and 2016–2018) indicates that many sites are characterized by an unfavorable conservation status [8]: (1) over 30% of the positions of the *Molinia* meadows located on calcareous, peaty, or clayey-silt-laden soils with fluctuating water tables (alliance *Molinion caeruleae*, code 6410); (2) 37% of extensively use mesophile, lowland hay meadows (alliance *Arrheratherion elatioris*, code 6510); (3) over 40% of the xerothermic, dry grasslands on calcareous substrates (class *Festuco-Brometea*, code 6210) and (4) over 60% of the locations of alluvial meadows of river valleys with a natural dynamic of flooding belonging to the *Cnidion dubii* alliance (code 6440). Only approx. 10% of *Cnidion* and xerothermic grasslands, 13% of *Molinia* meadows, and slightly more, i.e., 22% of *Arrheratherion* meadows have a favorable conservation status. Therefore, an important task is to improve the condition of grassland habitats, among others, by introducing species and restoration of multi-species meadows [9,10]. Actions taken to restore degraded and damaged grassland ecosystems are currently of particular importance, as the years 2021–2030 were defined by the decision of the UN General Assembly as the "UN Decade on ecosystem restoration" [11].

Restoration and recreation of species-rich semi-natural grasslands is difficult, because there are a number of factors limiting the course of this process, e.g., inadequate moisture conditions, inappropriate nutrient content and soil pH [12], significantly limited amount of diaspores in the soil seed bank [13,14], from dispersion, lack of diaspore carriers (migrating herds of animals), reduction of flooding, lack of ecological corridors and significantly limited initial plant development due to competition of old sward vegetation and the lack of suitable microhabitats for colonization, germination and survival of seedlings [15]. The restoration of a significant floristic diversity of semi-natural meadows often requires the introduction of diaspores of representative species that can be obtained directly from the donor meadow sward [16,17].

The successful establishment of introduced species depends on many features: morphological, biochemical, physiological, structural, phenological, and behavioral [18,19]. Functional traits influence the different stages of a species' life cycle, such as germination, expansion, and maintenance in the sward. Many plant species have seed dormancy mechanisms that prevent germination in conditions that are unfavorable to seedling survival. The conditions for germination analysis in accordance with the ISTA Rules are most comfortable for that process. The dormancy can be broken, for example, using the pre-chilling method. Temperature and humidity may limit seed germination, and tolerance to these factors may be species-dependent [20].

It has been found that a low mass of seeds and a large number of seeds can increase the probability of reaching a safe place for germination. Conversely, a large mass of seeds was identified as crucial for the success of introduction [21], as it provides nutrients in the germination phase, but success also depends on the number of seeds [22,23]. Thus, the success of species restitution depends on many features of diaspores and seedlings [24].

Despite the importance of generative reproduction of plants, little is known about diaspores and seed germination of many wild-flower native species, valuable meadow habitats, especially under varying weather conditions. For this reason, it is necessary to get to know them better. The research hypothesis was the assumption that, under favorable environmental conditions, plants develop even seeds with good germination capacity, while unfavorable conditions worsen the quality and increase the variability of the seed material characteristics. The main aim of the study was to link diaspore features (morphometric, mass) with the germination analysis. It was discussed how taking into account the characteristics of diaspores can improve the selection of plant species to restore valuable natural meadow communities. Answers were sought to the question of which features of the seed material can guarantee the success of the introduction. The results of the research on the size of diaspores and the germination capacity of seeds of the studied species may be useful in projects for the protection and restoration of valuable meadow communities.

2. Materials and Methods

2.1. Botanical Characteristics of Species

The research covered 28 species of natural flora, 4 non-forest natural habitats, protected by the Habitats Directive, occurring in the valleys of large rivers of interest to the community (EU) (Table 1):

- 2 species of *Cnidion* meadows (habitat type code 6440; All. *Cnidion dubii*);
- 4 species of *Molinia* meadows (code 6410; All. *Molinion caeruleae*);
- 11 species of extensively used lowland hay meadows (code 6510; All. *Arrhenatherion elatioris*);
- 5 species of xerothermic calcareous grasslands (code 6210; Cl. *Festuco-Brometea*);
- 6 remaining species, belonging to other phytosociological units, but often found also in the above-mentioned types of meadows.

Table 1. Botanical characteristics of species.

Habitat—Code/Species	Family	Durability [1]	Life-Form [2]	Diaspore
***Festuco-Brometea*—6210**				
Artemisia campestris L.	Asteraceae	B, S	C	fruit
Centaurea stoebe L.	Asteraceae	D, B	H	fruit
Eryngium planum L.	Apiaceae	B	H	fruit
Scabiosa ochroleuca L.	Dipsacaceae	B, D	H	fruit
Verbascum thapsus L.	Scrophulariaceae	D	H	seed
***Molinion caeruleae*—6410**				
Galium boreale L.	Rubiaceae	B	H	fruit
Iris sibirica L.	Iridaceae	B	G	seed
Lychnis flos-cuculi L.	Caryophyllaceae	B	H	seed
Sanguisorba officinalis L.	Rosaceae	B	H	fruit
***Cnidion dubii*—6440**				
Allium angulosum L.	Amaryllidaceae	B	G	seed
Cnidium dubium (Schkuhr) Thell.	Apiaceae	B	H	fruit
***Arrhenatherion elatioris*—6510**				
Achillea millefolium L.	Asteraceae	B	H	fruit
Campanula patula L.	Campanulaceae	D, B	H	seed
Centaurea jacea L.	Asteraceae	B	H	fruit
Daucus carota L.	Apiaceae	D	H	fruit
Geranium pratense L.	Geraniaceae	B	H	seed
Lathyrus pratensis L.	Fabaceae	B	H	seed
Leontodon autumnalis L.	Asteraceae	B	H	fruit
Plantago lanceolata L.	Plantaginaceae	B	H	seed
Rumex acetosa L.	Polygonaceae	B	H	fruit
Tragopogon orientalis L.	Asteraceae	D	H	fruit
Trifolium pratense L.	Fabaceae	B	H	seed
Others				
Armeria maritima (MILL.) WILLD	Plumbaginaceae	B	H	fruit
Hypericum perforatum L.	Hypericaceae	B	H	seed
Linaria vulgaris L.	Plantaginaceae	B	G, H	seed
Lysimachia vulgaris L.	Primulaceae	B	H	seed
Potentilla erecta (L.) RAEUSCH	Rosaceae	B	H	fruit
Veronica longifolia L.	Plantaginaceae	B	H	seed

[1] Durability: B—perennial, D—biennial, S—subshrub. [2] Life form: H—hemicryptophyte, G—geophyte; C—herbaceous chamaephyte.

The names of vascular plants are in accordance with those presented by Mirek et al. [25]. For each species, the following parameters were specified: family, biological-stability (durability), life-form using the Raunkiaer scale, the type of generative diaspores. These parameters were identified based on the following sources: Szafer et al. [26], Ellenberg and Leuschner [27], Cappers et al. [28].

The studied plant species belong to 17 botanical families, most of them are perennials, characterized by a similar way of adapting to environmental and climatic conditions (life forms). Three species are typical biennial plants, and three others; depending on the climatic conditions, they can be biennial or perennial (Table 1).

Most of the species in question are hemicryptophytes, i.e., plants whose buds are above the ground (winter buds are above or just below ground) and are protected by live or dead leaves, mulch, topsoil or snow cover. These species are more susceptible to freezing or prolonged drought than geophytes (cryptophytes with buds below the soil surface) (Table 1).

When analyzing the possibility of generative reproduction of the studied species (the structure of their generative diaspores), it was found that the spread of 15 species is ensured by single-seeded fruit (non-bursting), while typical seeds constitute propagation material in 13 species (Table 1).

2.2. Seed Collecting

The ripe diaspores (fruits and seeds) were collected by hand, in the third decade of September, in three successive growing seasons in the years 2015–2017, on the Special Habitat Protection Area—The Lower Pilica Valley (PLH 140016), near Mniszew (51°51′04.1″ N 21°15′57.2″ E, 51°50′00.3″ N 21°17′05.2″ E).

For each species, ripe seeds were taken from at least 2 populations of a minimum of 10 individuals of a single large population and mixed [29]. After cleaning (separation of the fully developed seeds from empty seeds, infected seeds, and the rest of the pericarp; by hand and using the laboratory sieves) the seeds were kept in paper bags under dry conditions at 15 °C for 5 months, after which the germination tests were started.

2.3. Morphometric Characteristic and Germination Capacity Analysis

The morphometric evaluation of the diaspores collected in 2016 and 2017 was performed, while the length, width, and thickness (30 seeds of each species) were determined using a binocular microscope equipment with an ocular micrometer. The weight of 1000 diaspores (TSW; 100 seeds each in 4 replications) was tested using a digital analytical balance with an accuracy of 0.0000 g.

The germination capacity analysis was carried out in accordance with ISTA Rules [30]. Three replications of 50 seeds were placed in plastic boxes on filter paper moistened with distilled water. Water was added to keep 60% of water maximum retention. The samples were put into the incubator at a variable temperature of 20/30 °C, i.e., 20 °C for 16 h (in the darkness) and 30 °C for 8 h with the light (750 to 1.250 lx). For breaking dormancy, half of the tested species needed pre-chilling 28 days in 7 °C (in darkness), and *Galium boreale* longer to 84 days [31]. Without pre-chilling were: *A. millefolium*, *A. maritima*, *A. campestris*, *D. carota*, *E. planum*, *G. pratense*, *H. perforatum*, *L. pratensis*, *L. autumnalis*, *L. flos-cuculi*, *P. lanceolata*, *S. ochroleuca*, *T. pratensis* and *V. thapsus*. The first estimation of seed germination was made after 7 days of the end of pre-chilling. Then, the seed samples were checked every two days for germinated seeds (emerged radicle), which if present were recorded and removed at each counting. The criterion for germination was a protruded radicle $\geq$2 mm. The last counting was made after 28–63 days, depending on the species. The analysis was made with the division into normal seedlings (showing all elements of the plant structure of a given species), abnormal seedlings (not showing all the elements of the plant structure, e.g., not having a properly developed root), dead seeds (not germinated but had a soft seed coat and/or was strongly infected by fungi) and fresh ungerminated seeds (FUS; not germinated, had a hard seed coat and was not infected by fungi).

2.4. Statistical Analyses

The statistical analysis of the research results was performed for two data sets. In the first of them, each of the 28 species (multidimensional observation) was described using the mean values of the parameters of the germination analysis. In the second data set, in addition to the results of the germination capacity analysis, the results of morphometry measurements were also included. The PCA method was used to search for regularities in the analyzed data sets. The PCA results [32] enable the identification of significant explanatory variables and observations describing the studied group of species. As part of the pre-processing, each of the explanatory variables, both data matrices, was transformed in order to obtain the mean value equal to zero and the variance equal to 1. The method of agglomeration hierarchical clusters was used to establish homogenous groups of species, using the Ward's algorithm using the square of the Euclidean distance [33].

The results of the analyses are presented in PCA diagrams illustrating the correlation of the explanatory variables to the first and second principal components. However, to assess the relationship between the explanatory variables and observations (species), the so-called biplots were used. The structure of homogenous groups is presented in the form of dendrograms. The results of this type of analysis make it possible to study the migration of individual observations between clusters obtained on the basis of hierarchical clustering of two data sets. The structure of statistically significant differences in homogenous groups was presented for the most important explanatory variables using box-plots. The significance of the differences was investigated using the non-parametric Wilcox test. Moreover, two-factor analyses of variance (ANOVA) were performed (1st factor—species, 2nd factor—year of study) The verification of the significance of differences was based on Tukey's confidence intervals ($\alpha = 0.05$). The calculations were performed using the computer program Statgraphics Plus, version 4.1 [34].

2.5. Weather Conditions

The weather conditions during the research period were highly differentiated (Table 2). The growing seasons in 2015 and 2016 were extremely dry and very dry, respectively, with $\leq$0.1 mm of precipitation per 1 °C (0.091 mm·°C^{-1} in 2015; 0.100 mm·°C^{-1} in 2016). Particularly unfavorable conditions prevailed in the period of flowering, fruit development, and seed maturation of most of the studied species in July (0.104 mm·°C^{-1}) and August (0.016 mm·°C^{-1}) 2015—respectively in a dry and extremely dry period [35], with long rainless periods. Moreover, in August this year, the temperatures were exceptionally high, and the average monthly air temperature in that month was 23 °C. September 2016 (0.02 mm·°C^{-1}) was also extremely dry. In 2016, only July was characterized by greater rainfall and can be described as quite wet (0.185 mm·°C^{-1}). On the other hand, the growing season in 2017 was wet, especially September was extremely humid (0.347 mm·°C^{-1}). Similarly to the previous year, July of this year was quite humid (0.186 mm·°C^{-1}), and August, similarly to the previous year, was quite dry (0.110 mm·°C^{-1}). Such extreme weather conditions were reflected in the diaspore size and seed germination capacity.

Table 2. Weather conditions in growing seasons 2015–2017.

Year	Month						Growing Season
	IV	V	VI	VII	VIII	IX	IV–IX
Mean temperature (°C)							
2015	9.1	13.7	18.1	20.5	23.0	15.6	16.7
2016	10.0	16.3	19.8	20.3	19.3	16.5	17.0
2017	8.0	14.9	18.9	19.2	20.0	14.1	15.8
Sum of precipitation (mm)							
2015	31.4	57.7	37.5	66.2	11.3	73.1	277.2
2016	34.2	23.3	56.9	116.5	71.7	9.8	312.4
2017	62.1	68.3	106.2	111.0	68.5	146.8	562.9
Characteristics of growing seasons				**Hydrothermal index of Vinczeffy (mm·°C^{-1})**			
2015	extremely dry			0.091			
2016	very dry			0.100			
2017	wet			0.194			

3. Results

3.1. Morphometric Characteristic of Species

The assessed species differed significantly in the size of the diaspores; essentially, tiny seeds. Diaspores over 3 mm long were only in 4 out of 28 species: *Tragopogon orientalis*, *Armeria maritima*, *Leontodon autumnalis* and *Iris sibirica*. Most species (17 species) were 1.2—2.7 mm long (Table 3). The smallest seeds—less than 1 mm long—were 7 species: *Artemisia campestris, Verbascum thapsus*, *Lychnis flos-cuculi*, *Campanula patula*, *Hypericum perforatum*, *Potentilla erecta* and *Veronica longifolia*. On average, the studied species of significantly larger diaspores (longer, wider and thicker) developed in 2016 compared to 2017. The greatest variability (the highest values of the coefficient of variation) of the assessed diaspore size parameters was characteristic for diaspore thickness (up to 50%), especially in 2017, and the smallest seed length (up to 23%).

Table 3. Diaspores morphometry (mm) of species and statistical results.

Species	Length			Width			Thickness		
	Year of Harvest (Y)								
	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
Artemisia campestris L.	0.79	0.66	0.72	0.38	0.36	0.37	0.17	0.12	0.14
Centaurea stoebe L.	2.81	1.84	2.32	1.16	0.83	0.99	0.78	0.61	0.69
Eryngium planum L.	3.28	2.20	2.74	1.32	0.93	1.12	0.90	0.52	0.71
Scabiosa ochroleuca L.	3.23	2.18	2.7	1.94	1.25	1.59	1.92	1.18	1.55
Verbascum thapsus L.	0.75	0.57	0.66	0.52	0.42	0.47	0.51	0.38	0.44
Galium boreale L.	1.87	1.29	1.58	1.67	1.06	1.36	1.22	0.83	1.02
Iris sibirica L.	3.12	3.20	3.16	2.74	2.50	2.62	1.45	1.43	1.44
Lychnis flos-cuculi L.	0.79	0.53	0.66	0.62	0.43	0.52	0.42	0.37	0.39
Sanguisorba officinalis L.	2.92	1.99	2.45	1.72	1.15	1.43	1.60	1.03	1.31
Allium angulosum L.	2.03	1.48	1.75	1.23	0.88	1.05	0.96	0.66	0.81
Cnidium dubium (Schkuhr) Thell.	2.05	1.71	1.88	1.28	0.99	1.13	0.84	0.61	0.72
Achillea millefolium L.	1.69	1.26	1.47	0.58	0.44	0.51	0.16	0.09	0.12
Campanula patula L.	0.47	0.31	0.39	0.32	0.18	0.25	0.16	0.10	0.13
Centaurea jacea L.	2.47	2.06	2.26	1.12	0.93	1.02	0.85	0.65	0.75
Daucus carota L.	2.78	1.80	2.29	1.28	0.85	1.06	0.64	0.44	0.54
Geranium pratense L.	3.01	2.35	2.68	1.76	1.48	1.62	1.51	1.31	1.41
Lathyrus pratensis L.	2.89	2.23	2.56	2.54	1.91	2.22	2.04	1.26	1.65
Leontodon autumnalis L.	4.53	3.43	3.98	0.47	0.45	0.46	0.41	0.33	0.37
Plantago lanceolata L.	2.33	1.75	2.04	1.04	0.75	0.89	0.56	0.44	0.50
Rumex acetosa L.	1.57	1.17	1.37	0.95	0.65	0.8	0.91	0.64	0.77
Tragopogon orientalis L.	9.09	3.20	6.14	1.08	0.15	0.61	1.04	0.13	0.58
Trifolium pratense L.	1.73	1.18	1.45	1.38	0.88	1.13	1.06	0.59	0.82
Armeria maritima (MILL.) WILLD	5.35	4.09	4.72	1.48	0.85	1.16	1.19	0.66	0.92
Hypericum perforatum L.	0.99	0.76	0.87	0.41	0.35	0.38	0.41	0.31	0.36
Linaria vulgaris L.	1.96	1.20	1.58	1.67	1.00	1.33	0.11	0.14	0.12
Lysimachia vulgaris L.	1.38	1.10	1.24	1.03	0.70	0.86	0.82	0.52	0.67
Potentilla erecta (L.) RAEUSCH	0.85	0.60	0.72	0.62	0.44	0.53	0.33	0.30	0.31
Veronica longifolia L.	0.72	0.52	0.62	0.51	0.35	0.43	0.05	0.12	0.08
$LSD_{0.05}$ (S)			0.404			0.134			0.133
Mean (Y)	2.41	1.67		1.17	0.83		0.82	0.56	
$LSD_{0.05}$ (Y)	0.057			0.019			0.019		

The thousand-seed weight (TSW) is a characteristic of the botanical genus and species. TSW ranged from less than 0.1 g to more than 11 g (Table 4). *Lathyrus pratensis*, *Iris sibirica*, *Geranium pratense*, and *Tragopogon orientalis* were characterized by the highest TSW (over 5 g on average). Most species (20) were characterized by small diaspores, with TSW ranging from 0.1 to 1.6 g. *Potentilla erecta*, *Artemisia campestris*, *Veronica longifolia* and *Campanula patula* stood out among the smallest diaspores with TSW below 0.1 g. In 5 species (*Achillea millefolium*, *Allium angulosum*, *Daucus carota*, *Lathyrus pratensis*, *Verbascum thapsus*) the seeds were even, their weight variation coefficient was below 10%. Significant variability in seed weight was observed in 5 species, including the highest for Iris sibirica (on average about 56%), *Tragopogon orientalis* (41%), *Hypericum perforatum* (34%), *Potentilla erecta* (31%) and *Geranium pratense* (25.7%). TSW varied considerably over the years of the study and, on average, for species significantly higher in 2017 compared to 2016.

Table 4. Thousand-seed weight (g) depending on species and statistical results.

Species (S)	Year of Harvest (Y)			CV (%)
	2016	2017	Mean	
Artemisia campestris L.	0.049	0.067	0.058	19.5
Centaurea stoebe L.	1.407	1.155	1.282	17.4
Eryngium planum L.	1.302	1.133	1.218	12.9
Scabiosa ochroleuca L.	1.963	1.625	1.794	16.8
Verbascum thapsus L.	0.103	0.117	0.110	9.2
Galium boreale L.	1.258	1.097	1.177	22.8
Iris sibirica L.	3.797	11.527	7.662	55.8
Lychnis flos-cuculi L.	0.105	0.130	0.118	21.5
Sanguisorba officinalis L.	1.347	1.790	1.568	17.7
Allium angulosum L.	1.337	1.283	1.310	8.2
Cnidium dubium (Schkuhr) Thell.	0.387	0.550	0.468	22.4
Achillea millefolium L.	0.095	0.103	0.099	9.3
Campanula patula L.	0.018	0.030	0.024	27.6
Centaurea jacea L.	1.343	1.489	1.416	11.7
Daucus carota L.	0.665	0.755	0.710	9.4
Geranium pratense L.	5.850	9.300	7.575	25.7
Lathyrus pratensis L.	12.293	11.154	11.724	7.2
Leontodon autumnalis L.	0.663	0.537	0.600	15.0
Plantago lanceolata L.	1.257	1.244	1.250	13.3
Rumex acetosa L.	0.403	0.378	0.390	11.1
Tragopogon orientalis L.	3.387	7.411	5.399	41.0
Trifolium pratense L.	1.183	1.255	1.219	14.0
Armeria maritima (MILL.) WILLD	1.080	1.177	1.128	10.1
Hypericum perforatum L.	0.072	0.133	0.102	34.1
Linaria vulgaris L.	0.137	0.120	0.128	12.5
Lysimachia vulgaris L.	0.271	0.320	0.295	24.2
Potentilla erecta (L.) RAEUSCH	0.080	0.052	0.066	31.0
Veronica longifolia L.	0.038	0.042	0.040	17.8
$LSD_{0.05}$ (S)			2.11	
Mean (Y)	1.496	1.999		
$LSD_{0.05}$ (Y)	0.29			

3.2. Germination Capacity Analysis

The basic feature of the seed material is the germination capacity expressed as the percentage of normally germinated seedlings during the analysis according to the ISTA regulations. The differentiation of this feature in the studied species is presented in Table 5. The obtained results indicate that 8 species had a germination capacity of over 70% on average. Among them there are representatives of all-natural habitats: *Lychnis flos-cuculi* (6410), *Allium angulosum* (6440), *Daucus carota* (6510), *Leontodon autumnalis* (6510), *Achillea millefolium* (6510), *Verbascum thapsus* (6210) and *Veronica longifolia* as well as *Lysimachia vulgaris* (species characteristic of the *Filipendulion*). Significantly lower germination capacity (on average less than 30%) was found in 7 species. They were: *Cnidium dubium* (6440), *Linaria vulgaris*, *Potentilla erecta* and *Armeria maritima*, *Geranium pratense* (6510), *Lathyrus pratensis* (6510), and *Trifolium pratense* (6510). The remaining 13 species had a germination capacity of 30–67%. The lowest (less than 10%) variability of this trait, on average, over the period of the study, was found in 7 species: *Artemisia campestris*, *Verbascum thapsus*, *Lychnis flos-cuculi*, *Daucus carota*, *Leontodon autumnalis*, *Plantago lanceolata* and *Lysimachia vulgaris*.

Table 5. The germination capacity (%) of seeds depending on species and statistical results.

Species (S)	Year of Harvest (Y)			Mean
	2015	2016	2017	
Artemisia campestris L.	65.3	64.0	70.7	66.7
Centaurea stoebe L.	10.7	44.0	36.0	30.2
Eryngium planum L.	48.0	41.3	71.3	53.6
Scabiosa ochroleuca L.	34.7	58.7	68.7	54.0
Verbascum thapsus L.	83.3	92.0	88.7	88.0
Galium boreale L.	34.0	60.0	38.7	44.2
Iris sibirica L.	87.3	38.7	72.7	66.2
Lychnis flos-cuculi L.	72.7	70.7	68.0	70.5
Sanguisorba officinalis L.	49.3	32.0	12.0	31.1
Allium angulosum L.	74.0	54.7	81.3	70.0
Cnidium dubium (Schkuhr) Thell.	2.7	18.7	44.0	21.8
Achillea millefolium L.	59.3	82.7	77.3	73.1
Campanula patula L.	31.7	64.0	18.0	37.9
Centaurea jacea L.	46.7	66.7	62.7	58.7
Daucus carota L.	74.0	74.7	88.7	79.1
Geranium pratense L.	17.3	22.7	19.0	19.7
Lathyrus pratensis L.	2.0	66.7	4.7	24.5
Leontodon autumnalis L.	64.7	82.7	79.3	75.5
Plantago lanceolata L.	69.3	69.3	61.3	66.6
Rumex acetosa L.	60.7	76.0	62.7	66.5
Tragopogon orientalis L.	14.7	40.0	96.0	50.2
Trifolium pratense L.	13.3	4.0	12.0	9.8
Armeria maritima (MILL.) WILLD	2.0	17.3	24.0	14.4
Hypericum perforatum L.	50.7	36.0	82.7	56.5
Linaria vulgaris L.	9.3	17.3	34.7	20.4
Lysimachia vulgaris L.	70.7	69.3	79.3	73.1
Potentilla erecta (L.) RAEUSCH	5.3	68.0	8.0	27.1
Veronica longifolia L.	73.3	85.3	82.0	80.2
$LSD_{0.05}$ (S)				28.88
Mean (Y)	43.8	54.5	55.2	
$LSD_{0.05}$ (Y)		5.93		

A large variety of species was found in terms of germination capacity depending on the course of weather conditions during flowering, fruit development and seed filling. The catastrophic drought in 2015 caused weak plant growth, weak earing, early ripening, and insufficient forming and fulfilling of the seeds and, consequently, reduced germination capacity, which was on average significantly lower than in the following years (Table 5).

Abnormal seedlings were the second fraction released during the germination analysis. The highest share of these seedlings, on average over 10%, was distinguished by only two species, i.e., *Hypericum perforatum* (21.1%) and *Artemisia campestris* (10.2%). In other species, they accounted for less than 5% on average. The seed material from the years with a significant rainfall deficit (2015 and 2016) was characterized by a significantly higher share of abnormal seedlings compared to the material from the wet year (2017). Thus, in 2016, there were as many as 56% of this fraction of seedlings in *Hypericum perforatum*. On the other hand, in *Artemisia campestris*, *Achillea millefolium* and *Veronica longifolia*, the highest share of abnormal seedlings was found in 2015 (22.7%, 12% and 10.7%, respectively). In 2017, the share of this fraction of seedlings in all studied species was small and accounted for less than 5.5%.

The drought also increased the share of dead seeds, which in 2015, on average for species, was significantly the highest compared to the remaining years of the study. This year, over 50% of dead seeds were found in as many as 12 species, including over 80% in 7 species: *Centaurea stoebe*, *Cnidium dubium*, *Lathyrus pratensis*, *Tragopogon orientalis*, *Armeria maritima*, *Linaria vulgaris* and *Potentilla erecta*.

The seed material obtained in the year with the highest total precipitation (2017) and exceptionally wet September was characterized by a significantly higher share of fresh ungerminated seeds (FUS). The greatest number of FUS (over 75%) this year was developed by species from the *Fabaceae* family (*Lathyrus pratensis*, *Trifolium pratense*) and *Geranium pratense* and *Campanula patula*. *Galium boreale* and *Plantago lanceolata* (45% and 35%, respectively) had a significant share of this seed fraction. Conversely, *Sanguisorba officinalis* and *Allium angulosum*, unlike the species mentioned above, had the highest share of fresh ungerminated seeds in 2016 (approx. 27% and 44%, respectively). It should be noted that the seed material of half of the studied species (14 species) did not contain dormant seeds at all, and *Campanula patula*, *Cnidium dubium,* and *Potentilla erecta* were characterized by their presence only in 2017.

3.3. Hierarchical Clustering and PCA Analysis of Seed Species

Cluster analysis performed for two data sets characterized by germination analysis data (set 1) and germination analysis data supplemented with diaspore morphometric data (set 2) are illustrated in the form of dendrograms, as shown in Figure 1. The binding distance shown in the tree diagrams is the square of the Euclidean distance, which represents the similarity between the clusters of the analyzed plant species. Differences in connection levels in clusters can serve as a means of distinguishing the number of clusters resulting from the analysis. As a result of the graphical analysis and the applied "Silhouette" test [33], the structure of four clusters marked with the following colors was obtained: red, orange, blue, and green.

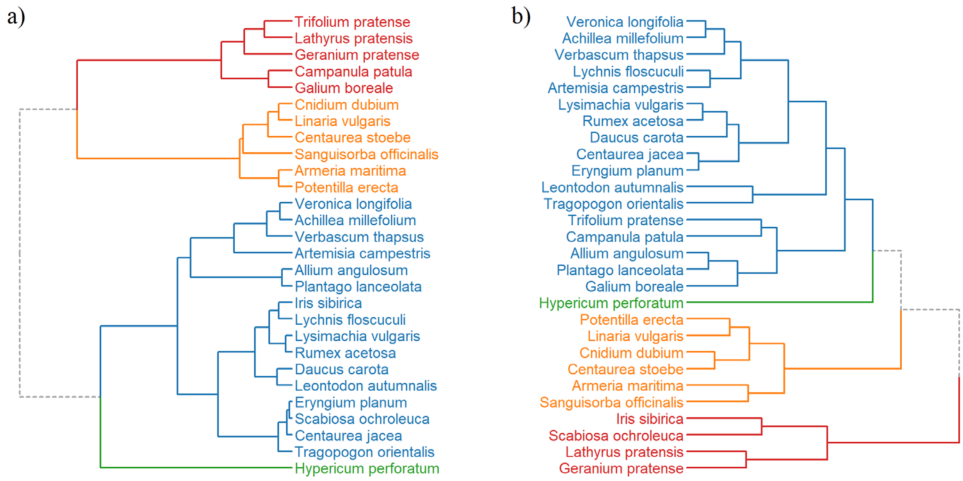

Figure 1. Agglomerative hierarchical clustering dendrograms of plant species for (**a**) dataset1 and (**b**) dataset2.

It is noteworthy that, regardless of the set of input data (set 1 and set 2) used in the cluster analysis, the same group of plants with similar parameters of germination analysis and morphometric features of diaspores (orange) was distinguished. This group is characterized, among others, by low germination capacity (average 14.4–31.1%). A characteristic feature of both developed dendrograms was the distinction of a separate group containing only one species, i.e., *Hypricum perforatum*. This was most likely related to the over-representativeness of one explanatory variable, i.e., abnormal seedlings. The largest group are species marked in blue. Regardless of the data set used in the analysis (set 1 and set 2), most species of this group occur in both dendrograms; however, it should be emphasized that supplementing the input data with variables describing the morphometry in the cluster analysis resulted in migration between the clusters marked in red and blue.

An attempt to explain the affiliation of individual observations to clusters is illustrated in the biplot PCA figure (Figure 2). The presentation of the data structure in this way (set 1 and set 2) showed that the separation of the orange group was mainly determined by one explanatory variable representing the share of deed seeds and it was independent of the size of the input data matrix. This means that the species mentioned in this group were characterized by a high percentage of dead seeds (67–85%). A similar observation applies to the green group (*Hypericum perforatum*), whose separation was determined by a high share of abnormal seedlings (21%). The blue groups include species with over 50% germination capacity values. The determination of the red group for the first set of data was mainly determined by the variable fresh ungerminated seeds (25–56%). However, after supplementing the input data with morphometric data (set 2), it was observed that the main element distinguishing this group was the width and thickness of the diaspores and the weight of a thousand seeds. Increasing the sample information resulted in several species migrating between the blue and red clusters. They were: *Trifolium pratense*, *Campanula patula* and *Galium boreale* characterized by a large share of fresh ungerminated seeds (Figure 2a) and *Scabiosa ochroleuca* and *Iris sibirica* species with wide and thick diaspores and relatively large TSW (Figure 2b).

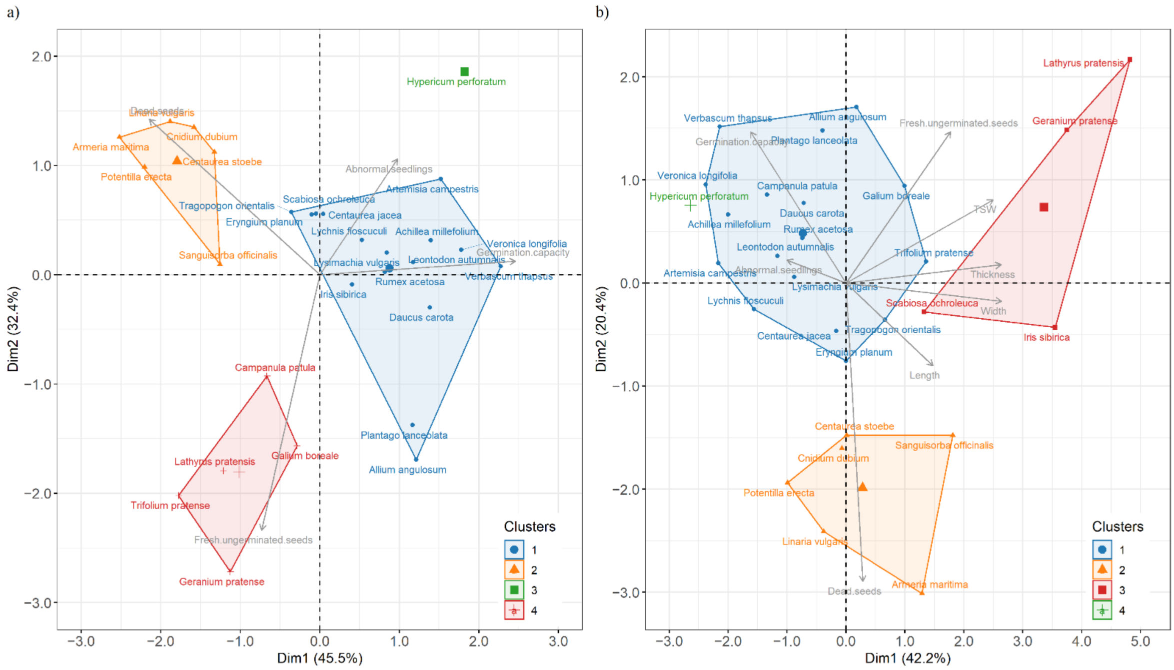

Figure 2. PCA biplots indicating distinguished groups of species and descriptive variables for (**a**) dataset1 and (**b**) dataset2.

The variance analysis performed for the full data set (set 2) allowed for the determination of significant differences between the selected groups of plants. The obtained results of this analysis were illustrated in the form of box-plots for the main explanatory variables (Figures 3 and 4). The results of the variance analysis showed quantitatively that the grouping of plants was significantly influenced by: high germination capacity and average values of the share of deed seeds and FUS. The morphometric data for this group of plants oscillated around the average, with the exception of the TSW trait. In the separated group (orange), a very low germination capacity can be observed, as well as a low share of FUS. Conversely, the share of deed seeds in this group was significantly higher than in the other groups and amounted to about 60%. The fact that the group of plants (red cluster) was distinguished was confirmed by significantly higher values of the morphometric features of diaspores compared to the other groups. Based on the analysis, it can be concluded that *Hypericum perforatum* is a candidate for the group of species with the highest germination capacity (56%); however, a large share of abnormal seedlings was most likely a feature that determined the separation of a separate group for this species.

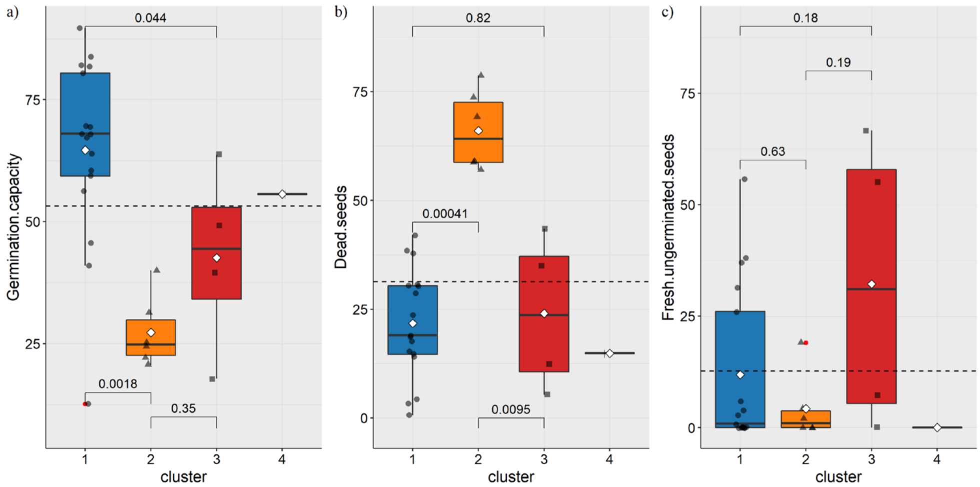

Figure 3. Boxplot of descriptive variable for distinguished clusters of the measured dataset2. (**a**) Germination capacity (**b**) Dead seeds and (**c**) FUS. The scattered point indicates the measured value characterizing certain plant species parameter. Dotted line indicates overall average of the measured variable. The diamond symbol indicates the average value of plant species variable for each cluster The red dots indicate the outliers.

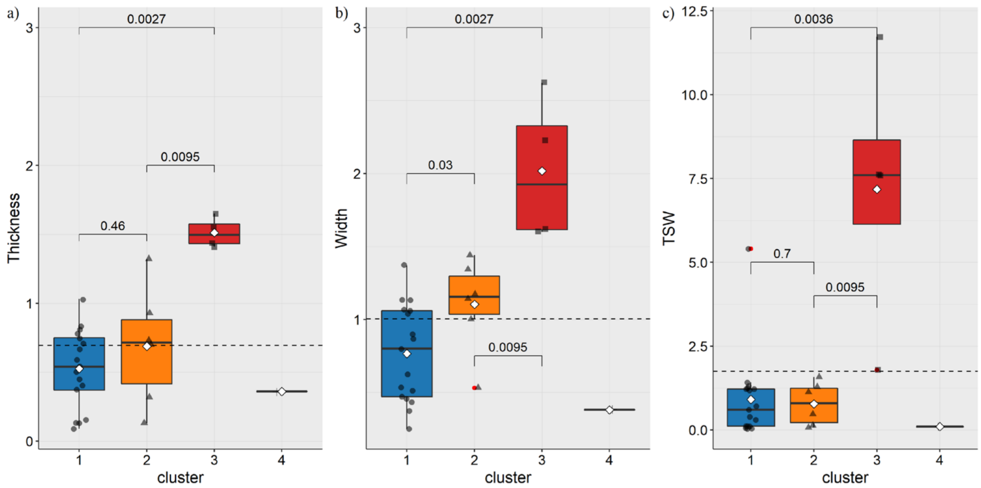

Figure 4. Boxplot of morphometric variables for distinguished clusters of the measured dataset 2. (**a**) Thickness (**b**) Width and (**c**) TSW. The scattered point indicates the measured value characterizing certain plant species parameters. The dotted line indicates the overall average of the measured variable. The diamond symbol indicates the average value of plant species variable for each cluster, while the red dots indicate the outliers.

4. Discussion

The plant species that the study applies to are characterized (except for 4 species) by small diaspores with TSW below 2 g. This is consistent with the reports of, among others, Török et al. [36], who found that short-lived herbaceous species have a much greater seed weight than herbaceous perennials. Taking into account the assessed morphometric features of seeds determining their weight, it should be emphasized that TSW depended to the greatest extent on the width and thickness of the seeds (correlation coefficient 0.620 and 0.602, respectively), while this relationship was not found with regard to the seed length. TSW is a characteristic of a botanical genus, species, variety and undergoes little change, as shown in this study (in 18 species less than 20%). The stability of the thousand-seed weight of other species was demonstrated, among others, by Borawska-Jarmułowicz et al. [37].

Seed weight is an important biological factor influencing the seed germination rate, seedling elongation, and initial plant development. The relationship between seed weight and seedling size has been reported for different species [38,39]. It was found that larger seedlings develop from larger seeds, which because of the greater amount of accumulated reserve materials, are better adapted to competing with other plant species in dense plant patches and have a greater chance of survival compared to seedlings of species with small seeds [40–42]. In the authors' own research, species with larger seeds and larger TSW (over 1.0 g), distinguished by a relatively high (over 60%) seed germination capacity, indicate a high probability of successful introduction. These include *Iris sibirica*, *Allium angulosum*, *Plantago lanceolata;* however, in the case of species with very fine seeds and TSW below 0.1 g, and low germination capacity (below 30%), e.g., *Campanula patula*, *Potentilla erecta*, the success of the introduction cannot be expected.

On the other hand, small seeds can germinate faster than large seeds, which can promote faster colonization of new sites, re-colonization of initial microhabitats that appear randomly and temporarily, e.g., revealing themselves in the spring, as the water level in the lake decreases, along with coastal alluvia [42]. Among the studied species, there are three species characterized by very fine seeds (less than 0.1 g) and, at the same time, high seed germination capacity (above 67%). These are: *Artemisia campestris* (6210), *Achillea millefolium* (6510) and *Veronica longifolia*. The high germination capacity of seeds will favor the successful introduction of these species; however, in favorable weather conditions and the presence of sufficiently large gaps in the sward limiting competition. This is confirmed by the good effects of the introduction of, among others, *Achillea millefolium* obtained in studies on increasing the floristic diversity of abandoned *Arrhenatherion elatioris* meadows [43].

The variation in the size of fruits and seeds of different species is genetically determined and, moreover, is exacerbated by environmental factors. A particularly variable feature is the thickness of the seeds, which depends on their filling, which is greatly influenced by weather conditions in the period from setting the seeds to full maturity. Better environmental conditions guarantee greater numbers and larger sizes of seeds and their lower variability. This suggests that species with low seed weight, such as: *Verbascum thapsus*, *Lychnis flos-cuculi*, *Campanula patula*, should be sown with material that allows for even distribution on the soil surface (e.g., with sand). This is also important because the fine seeds germinate better in the light than in the dark [44]. Taking into account the fact that the key species for the same habitat have diaspores of different sizes and masses, it can be assumed that the effectiveness of community restoration by the method based on the transfer of seed material collected from the donor area (without its division into species) may be lower than after sowing diaspores of individual species, as noted in the earlier work [45].

The results of the discussed own research showed that there is no direct translation of the size and TSW on the success of introduction expressed by the germination capacity of seeds (correlation coefficient −0.291). Both in the group of species with large and small diaspores, there were species with high and low germination capacity. Similarly, Moles and Westoby [46], on the basis of an analysis of the development of the population of 112 species in their natural conditions, found no relationship between seedling survival and the size (mass) of seeds in the germination phase (until emergence), while this relationship occurred in the period of seedling establishment (after emergence) and further initial development (till the tillering phase). A particularly strong positive relationship between these features was found in the first week after emergence. Large-seeded species turned out to be more tolerant to unfavorable habitat conditions during their initial development (including competition from primary sward species, strong shading, drought, nutrient deficiency, defoliation, herbivores) and showed greater survival than small-seeded species [46].

The value of the seed material is highly dependent on the weather conditions during the seed ripening period [47]. The seed germination assessment was carried out in the laboratory under strictly defined light, humidity, and temperature conditions, optimal for each species. Optimal conditions are rarely found in the field, usually, there are more or less stressful for germination. It has been shown that under more favorable environmental conditions, plants produce larger diaspore among others, Lehtilä and Ehrlén [48]. High temperature reduces the size of mature seeds [20]. In the presented research, extreme drought in 2015 resulted in both a reduction in seed weight and germination capacity and an increase in the share of dead seeds; it was found that germination capacity was negatively correlated with the presence of dead seeds ($r = -0.571$). The fungal spores on the surface of the seeds in conditions of adequate humidity strongly infected the seeds and caused them to die. Water supply also modifies the proportion of mature dormant seeds [20]. Segura et al. [49] found that seed dormancy in many species may be dependent on the environmental conditions in which the parent plants during seed development were grown. Seeds produced under stressful conditions, e.g., very dry weather with high temperature, have a lot of hard dormant seeds. Many such seeds are also produced in wet and cool years, and under such stressful conditions, the parent plants introduce seeds into the dormancy. This is confirmed by the negative correlation coefficient between germination capacity and the share of fresh ungerminated seeds ($r = -0.486$). Moreover, it was found that a larger share of fresh ungerminated seeds was positively correlated with the seed weight (TSW), the correlation coefficient between these features was $r = 0.432$. The highest share of fresh, ungerminated seeds occurred in the wet year (2017), while significantly lower in 2015 and 2016. This is consistent with the results of studies by Eslami et al. [50], who showed that seeds produced in cool and wet years often are dormant than those produced in dry, warm years. This also confirms previous reports that seeds that develop at high temperatures have less dormancy at maturity than those that develop at lower temperatures [20,51,52]. It should be noted that the more frequent weather anomalies in Poland (prolonged droughts, heavy rains) in connection with the progressive climate change will have an increasing impact on seed dormancy, changes in seed germination conditions, and the establishment of individual plant species. Eslami et al. [50] report that some species (e.g., *Raphanus raphanistrum*) have several mechanisms responsible for long-term seed dormancy. The fact that the germination capacity of species can be very variable from year to year indicates the need to test it before sowing, which was also emphasized by Kiss et al. [53].

The longevity of seeds is related to their ability to fall into dormancy, which can be divided into physiological, physical, combined, morphological, and morphophysiological [54]. There are no specific morphological features of seeds related to long viability, only a strong relationship between the structure of the seed coat or pericarp and viability has been proven. Physical dormancy is known to occur in species from 18 botanical families of angiosperms and is very common in Fabaceae, *Cistaceae, Geraniaceae, Malvaceae, Rhamnaceae* and *Sapindaceae* [20]. The results of our research confirmed the presence of physical dormancy in the studied species from the *Fabaceae* family (*Lathyrus pratensis* and *Trifolium pratense*) and *Geraniaceae* (*Geranium pratense*). When introducing these species, it is recommended to increase the amount of sowing. Falling in and out of dormancy occurs cyclically and covers the period of several or even a dozen years. The seeds in the soil ensure the continuity of the occurrence of individual plant species in a given habitat. Seed dormancy and soil seed bank are critical to plant regeneration strategies, especially in ecosystems where rainfall during the growing season is highly variable and unpredictable.

The germination capacity of seeds depends on many factors (edaphic, climatic, environmental) and depends on the dormant state of the seeds. The most important factor regulating the germination of non-dormant seeds is the availability of water, then the temperature, especially in the initial period, when plants are not yet competing for light and nutrients [55]. The germination of the seeds of most species requires a sufficient amount of light. Global climate change, many aspects of which include seed germination requirements, has led to geographic variability in seed germination and emergence across species [56]. The universal features that will ensure success in the introduction are viable seeds and a sufficiently moist substrate. Low temperatures during germination break dormancy, which has been known for a long time, and indeed, we have no influence on the weather conditions in which the seeds were grown (wet or dry year), but they explain the sowing value of the seed material. Then, the conditions in which the collected diaspores are stored are important, and the result will not be visible until the following year. Wild species (only such species were included in our research) have adaptive features to long life.

The seed biology of wild species is not sufficiently understood. Difficulties in using seeds of native species for sowing arise, among others, from insufficient information on seed biology, which was previously noted by, among others, Commander et al. [57]. There is no information on the relationship between the germination capacity and the effectiveness of the introduction of herbaceous plant species used in the restoration of meadow communities. The knowledge of the germination of seeds is needed to properly estimate the amount of seeds needed to recreate a multi-species meadow community. The seed germination capacity is assessed mainly in laboratory or greenhouse conditions [58,59]. According to the authors, like Kövendi-Jakó et al. [60], the results of this assessment obtained in laboratory conditions are of high value for predicting the success of introductions of species and can be used in works related to increasing the floristic diversity of endangered communities and restoring valuable meadow communities to estimate the necessary amount of seeds. In addition, to determine the best sowing method for different species, taking into account requirements for low-temperature stratification or other seed pre-sowing treatments [61]; however, it should be remembered that based on the assessment of the germination capacity of seeds of a single species, it is not possible to fully predict the behavior of species after sowing in a mixture, because co-sowing species with different characteristics may product different, even completely opposite results, which may be due to allelopathic and competitive interactions. This problem was noted, for example, by Oliveira et al. [62]. The seeds of the species used in the mixture should have similar requirements for germination, then they will germinate simultaneously.

5. Conclusions

Species characterized by a good seed germination capacity (on average above 65%), regardless of weather conditions during the generative development period, are mainly: *Verbascum thapsus*, *Veronica longifolia*, *Daucus carota*, *Plantago lanceolata*. Despite the small size of the diaspores, especially the first two species, they can guarantee the success of introductions to a greater extent than others. Sensitive species with a large proportion of dead diaspores (over 50% on average), i.e., *Armeria maritima*, *Linaria vulgaris*, *Potentilla erecta*, *Centaurea stoebe*, *Sanguisorba officinalis*, *Cnidium dubium*, show little suitability for introduction. The use of species from the *Fabaceae* (*Lathyrus pratensis*) and *Geraniaceae* (*Geranium pratense*) families for introduction, despite the relatively large size of diaspores, may be unreliable due to the high proportion of fresh ungerminated (hard) seeds and, therefore, low germination capacity. When introducing these species, it is recommended to increase the amount of sowing.

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