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Author-formatted, not peer-reviewed document posted on 15/12/2025

DOI: <https://doi.org/10.3897/arphapreprints.e182427>

The impact of shifts in precipitation and temperature during seed ripening on the germination dynamics of 23 meadow species

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ABSTRACT

Weather fluctuations can influence germination dynamics and pose a threat to the conservation of lowland hay meadow species. This study aims to assess the effects of temperature and precipitation changes on the germination performance of 23 studied species, divided into two harvest periods (July and late September/early October) of a two-year collection (2022-2023). The study classifies these species based on shared germination characteristics and identifies key parameters influencing germination performance. Germination tests were conducted in Petri dishes at 20 °C for 16 hours (dark) and 30 °C for 8 hours (light), with a light intensity of 1000 lux. Quantitative (GC - germination capacity, FUS - fresh ungerminated seeds, and DS - dead seeds) and qualitative (t_{50} – the half of germination time, T. Index - Timson index) aspects were analysed using two-way ANOVA. Results revealed significant interactions between species and weather conditions across both collection periods. Wetter conditions increased GC and shortened t_{50} associated with reduced germination velocity in most species, while warmer and drier conditions prolonged t_{50} and increased DS share. Heatmap clustering revealed that several species, including *Tragopogon pratensis*, *Dianthus deltoides*, and *Centaurea jacea*, demonstrated stable germination performance, making these species promising candidates for restoration. The decision tree analysis identified the Timson index as the most influential parameter explaining GC. These findings demonstrate that understanding germination dynamics altered by climate change may aid conservation-oriented restoration efforts for local species, enabling the implementation of seed mixtures of introduced species and informed decisions on the optimal time for seed recruitment.

Keywords: *Arrhenatherion* meadows, Half of germination time, Germination dynamics, Germination capacity, Maternal effects, Seed mixtures, Timson index

1. INTRODUCTION

Grasslands, both natural and semi-natural, are one of the major habitats that support numerous ecosystem services, including water supply and regulation, carbon storage, erosion prevention, climate change mitigation, and pollination (Bengtsson et al., 2019). The multifunctionality of grasslands has resulted, among others, from the diversity of plant communities, such as those found in Lowland hay meadows, which are listed in Annex 1 of the Habitat Directive as protected habitats for the EU biodiversity target (European Commission, 2021; Habel et al., 2013). This type of meadow comprises 36 plant communities (associations) grouped into four alliances occurring in Western and Central Europe (Rodríguez-Rojo et al.

2017). Unfortunately, across Europe, many grasslands have suffered from abandonment, land-use change, and climate change, resulting in a low conservation status (Schils et al., 2022). For example, the conservation status of lowland hay meadows in many locations in Poland has deteriorated over the last decade and is now inadequate or unfavourable, as their plant communities are sensitive to fluctuating weather conditions (Janicka et al., 2021).

The frequent occurrence of extreme weather events (droughts and floods) has exacerbated anthropogenic impacts, reinforcing gradual or even rapid changes in land management in semi-natural grasslands (Shipley et al. 2024). Moreover, monthly temperature fluctuations in Europe are projected to increase by 1.5 to 4°C by 2080, accompanied by shifts in rainfall patterns (IPCC, 2014). Consequently, plants should rapidly adapt to maintain habitat resilience in the face of unpredictable adverse weather conditions (Veselá et al. 2020). Changes in temperature and rainfall intensity can significantly affect soil moisture and relative humidity over large areas, directly affecting the establishment requirements of plant populations, such as germination and seedling growth (Carta et al. 2022). One of the negative effects is the change in the proportion and time of seed germination inherited by seed progeny through mother plants, known as the maternal effect (Dudney et al. 2017).

Shifts in germination behaviour can gradually alter the fitness of plant communities and the capacity of the regeneration niche over time (Braz et al. 2016). For local native plants, changes in the germination niche shape germination dynamics (Pedrini and Dixon, 2020). However, previous studies of meadow *Arrhenatherion* species have shown that the use of germination dynamics as a tool for selecting target species for restoration has not been fully explored due to the high species diversity (Janicka et al., 2021). Meanwhile, the implementation of conservation-oriented restoration in response to climate change highlights the natural value of native species (Volis, 2019).

Nowadays, global restoration is facing limitations in preserving the gene pool of local species, making the need for restoration linked to nature conservation crucial (Toma et al. 2023). Although the importance of local species for conservation purposes in restoring degraded habitats is often overlooked due to species-specific reasons and environmental influences that affect seed germinability during seed maturation (Barr et al. 2019). Seed maturation plays a role in controlling the germination of progeny, as mature seeds can inherit germination-linked genes from mother plants that were activated under specific germination requirements (Chahtane et al. 2017). Thus, assessing the germination performance of local species should incorporate multiple aspects, including the number of germinated seeds and

quality aspects (germination time and speed), which reveal the maternal effect in progenies and help overcome this effect in the field (Pedrini and Dixon, 2020).

In this paper, our study fills an important knowledge gap regarding the germination dynamics of meadow *Arrhenatherion* species, driven by maternal effects, and changes in germination efficiency under variable climatic conditions, for conservation and restoration purposes. Therefore, this study has three objectives: (1) to assess the effect of weather variability between species from two harvest years on germination performances, (2) to classify species based on common germination characteristics, and (3) to identify key parameters influencing germination performances through decision tree analysis. This study also hypothesizes that: (1) seasonal weather variations in different harvest years influence the germination dynamics of species, (2) species with similar germination performance tend to belong to the same specific group, and (3) germination performance does not depend equally on all aspects of germination but may rather be determined by a specific aspect of the germination quality and quantity.

2. MATERIALS AND METHODS

Temperature and precipitation monitoring

The meteorological data included temperature and precipitation over four months in 2022 and 2023 (Fig. 1). The period for weather recording was determined by the seed maturation period of the studied species, which was at least a month prior to the collection time.

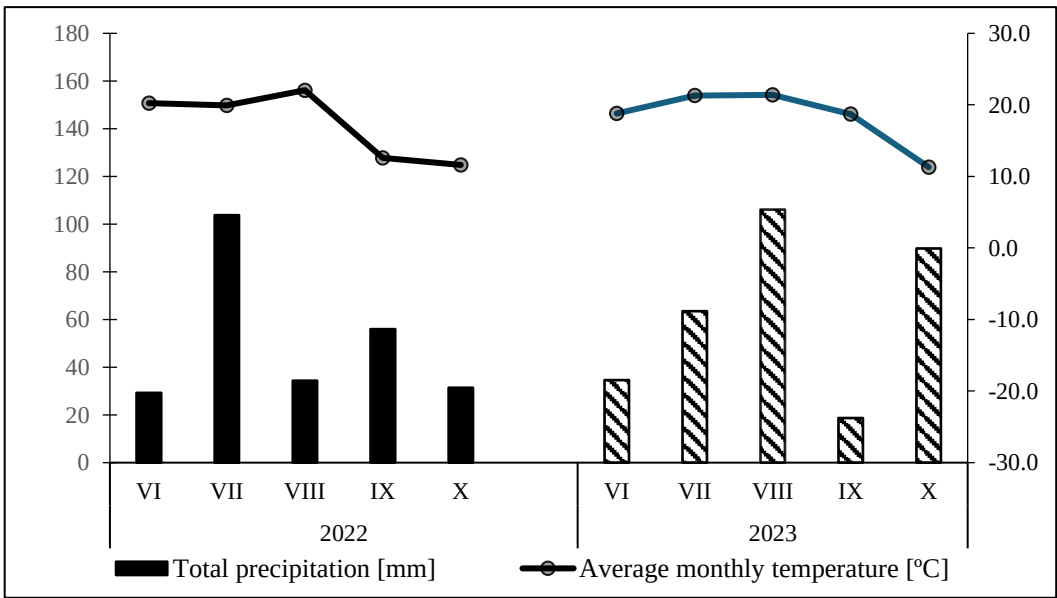


Figure 1. Meteorological data: Bar plots referred as total precipitation (mm) and line plots referred as average monthly temperature (°C) from June to October for the years 2022 and 2023

Study sites and Seed collection

Mature seeds of 23 studied meadow species were collected from three different meadows in the Mazovian Voivodeship (Table 1). Harvests were carried out in July 2022 and 2023 (14 species) and in early October 2022 or late September 2023 (9 species). The seeds were then cleaned of inert matter and other debris particles, stored in paper bags, and kept at approximately 20°C (room temperature) until the germination test was conducted.

Table 1. Taxonomy, time, and place of collection of the 23 studied meadow species

Species name	Species abbreviation	Botanical family	Collection time	Location and geographical coordinates
<i>Achillea millefolium</i> L.	Am	<i>Asteraceae</i>	September/October	Mniszew (Long 51.85 Lat 21.267)
<i>Allium angulosum</i> L.	Aa	<i>Amaryllidaceae</i>	September/October	Mniszew (Long 51.85 Lat 21.267)
<i>Alopecurus pratensis</i> L.	Ap	<i>Poaceae</i>	July	Mniszew (Long 51.85 Lat 21.267)
<i>Arrhenatherum elatius</i> (L.) P.Beauv. ex J.Presl & C.Presl	Ae	<i>Poaceae</i>	July	Mniszew (Long 51.85 Lat 21.267)
<i>Briza media</i> L.	Bm	<i>Poaceae</i>	July	Tymoteuszew (Long 52.25 Lat 21.65)
<i>Campanula patula</i> L.	Cp	<i>Campanulaceae</i>	July	Tymoteuszew (Long 52.25 Lat 21.65)
<i>Centaurea jacea</i> L.	Cj	<i>Asteraceae</i>	September/October	Tymoteuszew (Long 52.25 Lat 21.65)
<i>Dianthus deltoides</i> L.	Dd	<i>Caryophyllaceae</i>	July	Mniszew (Long 51.85 Lat 21.267)
<i>Eryngium planum</i> L.	Ep	<i>Apiaceae</i>	July	Mniszew (Long 51.85 Lat 21.267)
<i>Galium boreale</i> L.	Gb	<i>Rubiaceae</i>	September/October	Mniszew (Long 51.85 Lat 21.267)
<i>Geranium pratense</i> L.	Gp	<i>Geraniaceae</i>	September/October	Mniszew (Long 51.85 Lat 21.267)
<i>Hypericum perforatum</i> L.	Hp	<i>Hypericaceae</i>	September/October	Mniszew (Long 51.85 Lat 21.267)
<i>Kadenia dubia</i> (Schkuhr) Lavrova & V.N.Tikhom.	Kd	<i>Apiaceae</i>	September/October	Mniszew (Long 51.85 Lat 21.267)
<i>Knautia arvensis</i> (L.) Coult.	Ka	<i>Caprifoliaceae</i>	July	Tymoteuszew (Long 52.25 Lat 21.65)
<i>Lathyrus pratensis</i> L.	Lp	<i>Fabaceae</i>	July	Solec (Long 52.05 Lat 21.083)
<i>Leucanthemum vulgare</i> Lam.	Lv	<i>Asteraceae</i>	July	Mniszew (Long 51.85 Lat 21.267)
<i>Plantago lanceolata</i> L.	Pl	<i>Plantaginaceae</i>	July	Mniszew (Long 51.85 Lat 21.267)
<i>Sanguisorba officinalis</i> L.	So	<i>Rosaceae</i>	September/October	Mniszew (Long 51.85 Lat 21.267)

<i>Scorzoneroide autumnalis</i> (L.) Moench	Sa	<i>Asteraceae</i>	July	Solec (Long 52.05 Lat 21.083)
<i>Silene flos-cuculi</i> (L.) Greuter & Burdet	Sf	<i>Caryophyllaceae</i>	July	Mniszew (Long 51.85 Lat 21.267)
<i>Succisa pratensis</i> Moench	Sp	<i>Caprifoliaceae</i>	September/ October	Tymoteuszew (Long 52.25 Lat 21.65)
<i>Tragopogon pratensis</i> L.	Tp	<i>Asteraceae</i>	July	Solec (Long 52.05 Lat 21.083)
<i>Vicia angustifolia</i> L.	Va	<i>Fabaceae</i>	July	Mniszew (Long 51.85 Lat 21.267)

Note: Species names were given according to the Royal Botanic Garden, Kew, UK (2025) ([Plants of the World Online | Kew Science](#), accessed on 28.07.2025).

Germination test

Based on the International Seed Testing Rules (ISTA, 2018), the germination test was performed under a specific photoperiod regime (20 °C for 16 hours (darkness) and 30 °C for 8 hours (light); 1000 lux under an LED lamp). One hundred fifty seeds were used for each species, which were divided into three replicates of 50 seeds each (3 repetitions by 50 seeds). Seeds were set up in 94 mm plastic Petri dishes containing moistened Whatman No. 1 filter paper soaked in 1.5 mL of distilled water. After that, plastic Petri dishes containing seeds were secured with parafilm and placed in the versatile environmental test chamber (PHcbi Model no.MLR-352H-PE; Made in Japan). The test was conducted from the end of January to March 2023 (collection in 2022) and from the end of November to January 2024 (collection in 2023). Germination counts were recorded every other day for 34 days, during which germinated seeds were extracted after counting. Germinated seeds were identified by a visible radicle protrusion (1 mm) observed by the eye or under a magnifying glass (ISTA, 2018).

Formula for germination characteristics

GC

ISTA (2018) defines the percentage of seeds in a batch or replicate capable of germinating under optimal conditions as germination capacity (GC), as follows:

$$GC = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds tested}} \times 100\% \tag{1}$$

FUS and DS

According to ISTA (2018), the FUS embryo remains viable and green, without any decay or infection by fungi or other seed-borne pathogens. On the other hand, DS is characterized by a soft seed coat, as the decomposition of the embryo inside the seed by fungi or other seed-

borne pathogens renders the embryo inviable. The formula for both traits is similar to the formula for germination capacity, with a change in the number of FUS or DS.

The half-time of germination (t_{50})

Total germination days means the time span or duration from initial germination to its completion. The initial germination time refers to the point in time when the seed first sprouts. According to Farooq et al. (2004), the time required to obtain 50% of the number of seeds germinated is called the half-time of germination or t_{50} , and has the formula:

$$t_{50} = T_i + \frac{(\frac{N+1}{2} - n_i)(t_j - t_i)}{n_j - n_i} \quad (2)$$

Where: N - the final number of germinating seeds, and n_i and n_j - the cumulative number of seeds germinated by adjacent counts at specified t_i and t_j , respectively, when $N_i < \frac{N}{2} < N_j$.

Timson index (T. Index)

We used the modified Timson Index formula proposed by Khan and Ungar (1984) to calculate the germination velocity (rate). This index quantifies the germination rate, with higher values indicating faster germination, and its value is defined as a percentage (%):

$$T. Index = \sum \frac{G}{T} \quad (3)$$

Where: G - percentage of seeds germinated in a specific time interval, and T - total number of germination days.

Data analysis

R Studio 4.4.1 and Microsoft Excel were used to analyse germination data. All studied species were divided into two groups based on the month of collection to enable further analysis: 14 species (collected in July) and 9 species (collected in late September or early October). All germination parameters were calculated by loading the “germination metrics” package in R Studio. First, two-way analysis of variance (ANOVA) was used to evaluate germination dynamics in terms of quantity (GC, FUS, and DS) and quantitative aspects (t_{50} and T. Index). Parameters expressed as percentages (GC, FUS, DS, and T. Index) were transformed to arcsine form to meet the requirements of normality and homoscedasticity. Species was the first factor, and weather data was the second. In case of significant interaction effects, LSD (Least Significant Difference) post hoc tests were used. The “agricolae”, “emmeans”, “multcomp”, and “multcompView” packages were used for the analysis. Clustering plots in the form of heat maps with standardized Z-scores were then developed to examine species migration within groups based on changes in germination efficiency. This analysis was

visualized using the “pheatmap” package. To select the most important germination trait and the corresponding species, a decision tree analysis was developed and visualized using the “rpart” and “rpart.plot” packages. For species collected only in 2023 (July and September), two decision tree analyses were performed because the 2023 collection year was the priority in composing seed mixtures for the field experiment.

3. RESULTS

a. Interaction of species with weather conditions during seed ripening

The seed germination properties of the tested species were analysed using two-way analysis of variance (ANOVA). The interplay of species with various weather conditions is presented in Table 2 (for July collection) and Table 3 (for September/October collection). Both tables indicate that all seed germination parameters depend on the interacted factors

Table 2. Two-way analysis of variance (ANOVA) of seed germination parameters for 14 species collected in July 2022 and 2023

Parameter	Species		Weather		Species: Weather	
	Df	<i>p</i> -value	Df	<i>p</i> -value	Df	<i>p</i> -value
GC	13	< 2E-16***	1	7.98E – 12***	13	< 2E-16***
FUS	13	< 2E-16***	1	4.85E – 14***	13	< 2E-16***
DS	13	< 2E-16***	1	< 2E-16***	13	< 2E-16***
<i>t</i> ₅₀	13	4.01E – 07***	1	0.0022**	13	0.02*
T. Index	13	< 2E-16***	1	6.48e – 11***	13	< 2E-16***

Explanations: GC – Germination Capacity, FUS – Fresh Ungerminated Seed, DS – Dead Seed, *t*₅₀ – the median germination time, T. Index – Timson Index. Df – Degree of freedom, *p*-value is significant at **p*<0.05; ***p*<0.01; ****p*<0.001.

Table 3. Two-way analysis of variance (ANOVA) of seed germination parameters for 9 species collected in September/October 2022 and 2023

Parameter	Species		Weather		Species: Weather	
	Df	<i>p</i> -value	Df	<i>p</i> -value	Df	<i>p</i> -value
GC	8	< 2E-16***	1	0.609 ^{ns}	8	1.21E - 07***
FUS	8	1.06E - 11***	1	0.292 ^{ns}	8	0.0003***
DS	8	1.25E - 12***	1	0.613 ^{ns}	8	2.71E - 05***
<i>t</i> ₅₀	8	0.00013***	1	0.967 ^{ns}	8	0.002**
T. Index	8	< 2E-16***	1	0.739 ^{ns}	8	1.25E - 10***

Explanations: GC – Germination Capacity, FUS – Fresh Ungerminated Seed, DS – Dead Seed, t_{50} – the median germination time, T. Index – Timson Index. Df – Degree of freedom, ns – non-significant at $p<0.05$; p -value is significant at $*p<0.05$; $**p<0.01$; $***p<0.001$.

The obtained post hoc LSD results (Fig. 2A) for 14 species collected in July indicate that *Poaceae* species (*A. elatius*, *A. pratensis*, and *B. media*) showed the opposite trend, with *A. elatius* having a remarkable decrease in GC share (from approximately 86% in 2022 to 4% in 2023). Three species: *T. pratensis*, *S. autumnalis*, and *S. flos-cuculi* showed a slight increase, while the remaining species collected in 2023 exhibited a significant increase in GC, except for *A. elatius*, *C. patula*, and *L. vulgare*. Interestingly, three grass species also showed the highest proportion of FUS ($\pm 90\%$ for *A. elatius*, and $\pm 75.4\%$ for *A. pratensis*, and *B. media*) in the 2023 collection (Fig. 2B). However, seeds of two legumes (*L. pratensis* and *V. angustifolia*) harvested in 2023 showed a significant decrease in the FUS share ($\pm 73\%$ for both) compared to the 2022 harvest (approx. 27 and 24%, respectively). Most species collected in July decreased their DS share in the 2023 collection (Fig. 2C), except for *L. pratensis*, for which the DS share increased (from less than 25% to more than 40%), indicating that this phenomenon was influenced by weather factors.

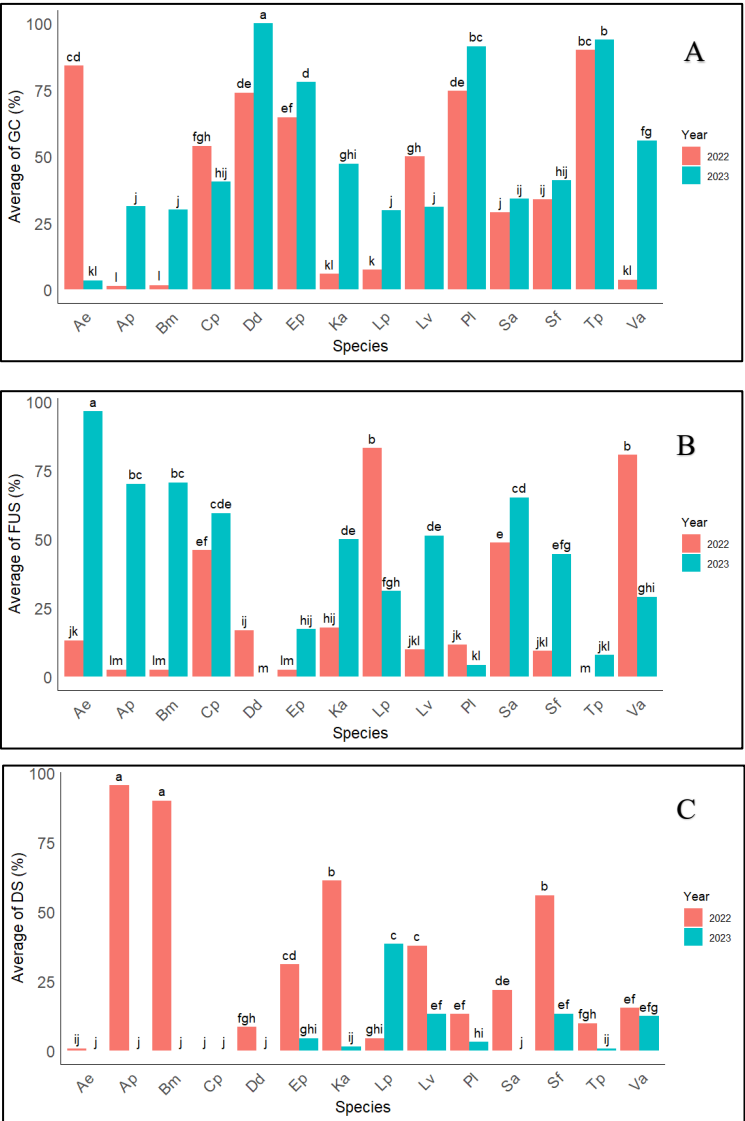
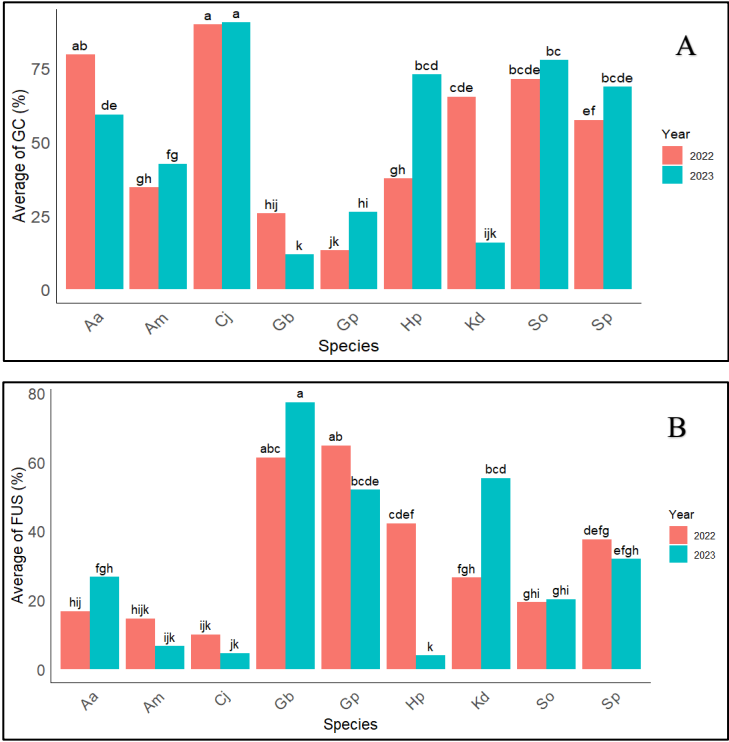


Figure 2. Quantitative germination characteristics of the species collected in July. A) Germination Capacity (GC in %), B) Fresh Ungerminated Seeds (FUS in %), and C) Dead Seeds (DS in %). The same letter indicates non-significant differences according to the LSD post hoc test at $p < 0.05$. The full names of the species are given in Table 1.

Among seeds collected in September/October, the greatest significant increase in GC was observed in *G. pratense* and *H. perforatum*, collected in 2023 (13.3% and 38%, respectively), compared to 2022 (28% and 72.7%, respectively). *C. jacea* seeds represented the highest GC, which was at a similar level in both harvest years (Fig. 3A). In contrast to other species, seeds of *A. angulosum* (59.3%), *G. boreale* (12%), and *K. dubia* (16%) had a noticeable decrease in GC in the 2023 harvest year. Regarding the share of FUS (Fig. 3B), a reciprocal trend was observed between *H. perforatum* and *K. dubia* in both years of study, with a significant decrease in the share of FUS in *H. perforatum* seeds (42.6% in 2022 and 6% in 2023). In the case of *K. dubia*, the opposite trend was observed, with a significantly higher share of FUS in seeds from the 2023 collection (55.3%) compared to seeds from the 2022 collection (26.7%). The most significant increase in the share of DS (Fig. 3C) occurred in the case of seeds of three species (*A. angulosum*, *C. jacea*, and *K. dubia*) obtained in 2023, by approximately 13.3%, 5.3%, and 28.7%, respectively. Conversely, the proportion of DS in the remaining three species (*H. perforatum*, *S. officinalis*, and *S. pratensis*) was significantly lower in 2023, amounting to 1.3%, 2%, and 0%, respectively.



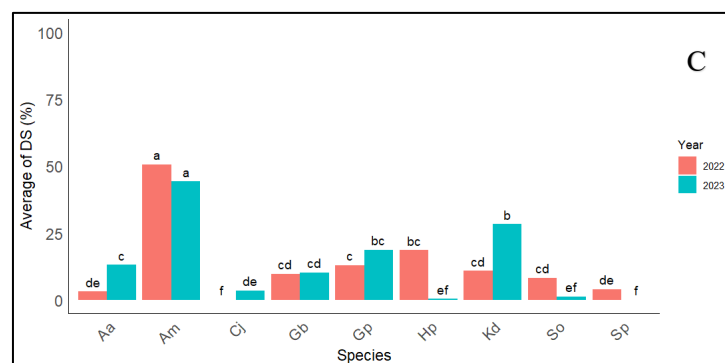


Figure 3. Quantitative germination characteristics of the species collected in September/October. A) Germination Capacity (GC in %), B) Fresh Ungerminated Seed (FUS in %), and C) Dead Seed (DS in %). The same letter indicates non-significant differences according to the LSD test at $p < 0.05$. The full names of the species are given in Table 1.

Concerning the aspects of seed germination quality, including t_{50} and Timson Index, varied by species and harvest year in both months. For instance, the t_{50} of seeds collected in July 2022 was longer than in 2023 (Fig. 4A) except for two grasses (*A. elatius* and *A. pratensis*) and one legume (*V. angustifolia*). Only two species had a dramatically reduced t_{50} in 2023 compared to 2022: *B. media* (from 16 to 5.9 days on average) and *K. arvensis* (from 16 to 6.7 days on average). In general, a longer t_{50} implies slower germination and vice versa, as represented by *A. elatius* with a significant decrease in the Timson index (from 36.9% per day in 2022 to 1.4% per day in 2023), followed by a moderate decrease in *L. vulgare* (from 21.6 to 14.7% per day) and last in *C. patula* (from 14.1 to 11.9% per day). The weather shift in 2023, primarily in June before the harvest in July, affects the faster T. Index compared to other harvests (Fig. 4B).

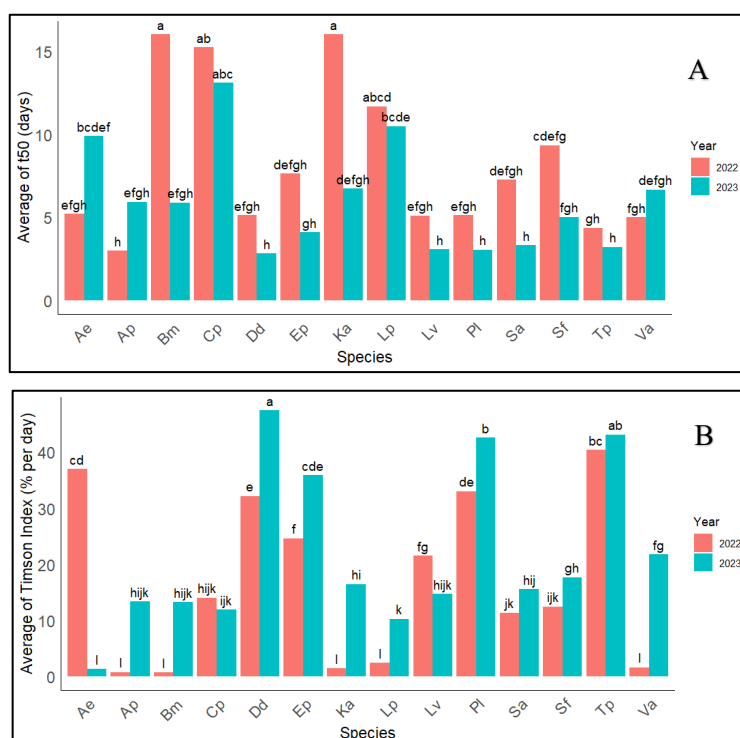


Figure 4. LSD bar plots of seed germination quality aspects of species collected in July 2022 and 2023. (A) t_{50} or median germination time (days), and (B) Timson Index (% per day). The same letter indicates non-significant differences according to LSD test at $p<0.05$. The full names of the species are given in Table 1.

Seeds of three species, such as *A. millefolium*, *G. pratense*, and *K. dubia*, performed a shorter t_{50} in the 2022 collection, while seeds of most species showed a shortened the t_{50} in 2023 (Fig. 5A). As observed, seeds of *G. boreale* recorded the greatest reduction of t_{50} in 2023 (from 16.3 to 10.2 days), in contrast to seeds of *G. pratense*, which recorded the greatest extension of t_{50} in the same year (from 6.8 to 19.5 days). Nevertheless, the seeds of these species were neither the fastest nor slowest in the Timson Index, as did the seeds of species collected in July (Fig. 5B). Instead, higher rainfall with lower temperature in September 2022 (compared to 2023) resulted in faster germination rates for *K. dubia* seeds (T. Index 27.9% per day) and *A. angulosum* (31.6% per day), but the following year (2023) seeds of both species collected under drier and warmer conditions responded with significantly slower germination (4.3% per day and 24.5% per day, respectively). Additionally, the Timson Index of *H. perforatum* seeds in 2022 (12.5% per day) was more than twice as low as that of 2023 seeds (30.9% per day), indicating a significant change compared to other species, with rather small variations between the years studied.

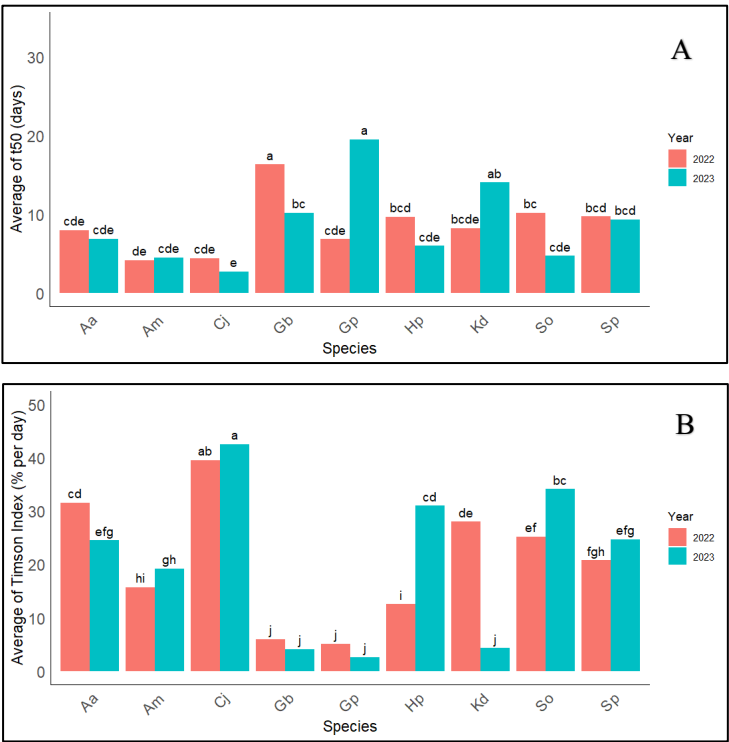


Figure 5. LSD bar plots of seed germination quality aspects of species collected in early October 2022 and late September 2023. (A) t_{50} or the median germination time (days), and (B)

Timson Index (% per day). The same letter indicates non-significant differences according to LSD post hoc at $p<0.05$. The full names of the species are given in Table 1.

b. Heatmap clustering seed germination parameters

Based on the z-score values in the heatmap, the germination performance in July 2022 (Fig. 6A) shows higher variability, highlighted by four species (*T. pratensis*, *A. elatius*, *D. deltoides*, and *P. lanceolata*) that have similarities of higher GC, faster T. Index, lower proportion of FUS and DS, and shorter t_{50} than others. In turn, species such as *L. pratensis* and *V. angustifolia* are grouped according to the highest content of FUS. Nevertheless, a longer t_{50} period, accompanied by a slower T. Index, was observed in *B. media* and *K. arvensis*. Compared to July 2023, this variability resulted in more uniform heatmap clustering, as some species showed changes in their germination performance (Fig. 6B). *E. planum* changed the position of *A. elatius* in the group of species with the highest GC and the fastest T. Index. Increasing monthly precipitation and decreasing temperatures led to a decrease in the proportion of DS and FUS in most species collected in July 2023, except *L. pratensis* and *A. elatius*.

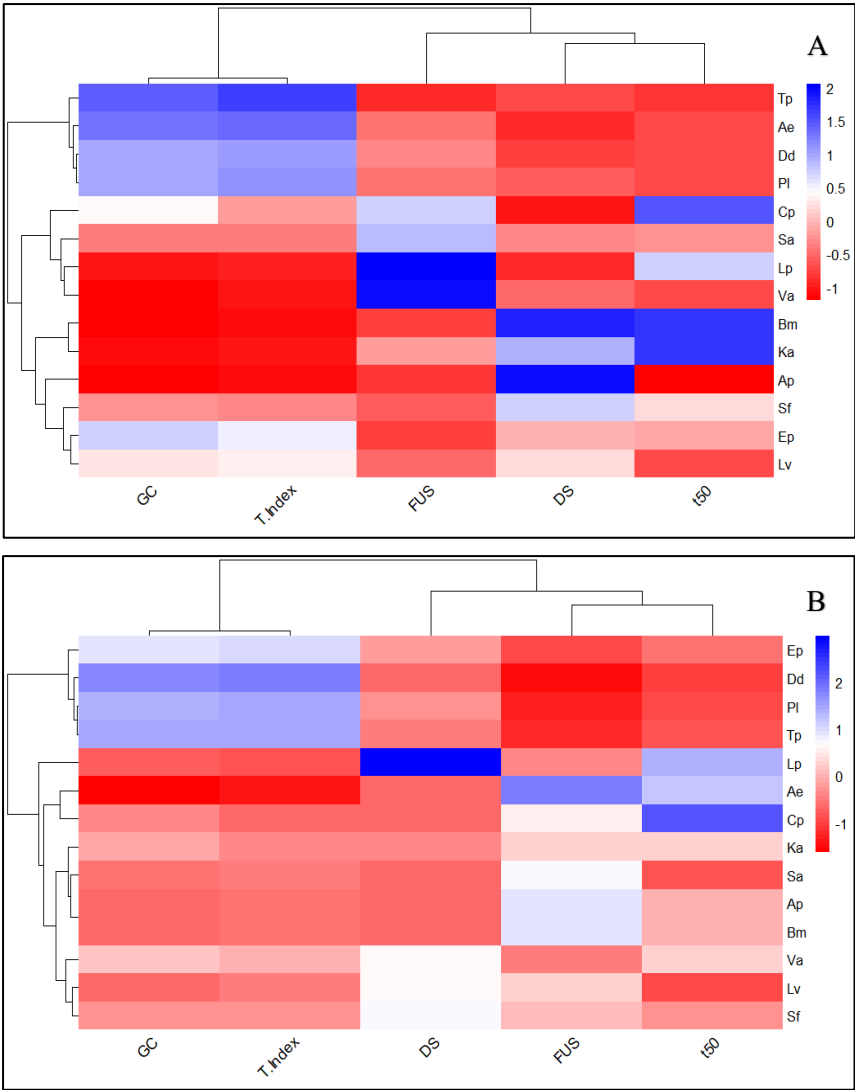


Figure 6. Heatmaps of the analysed germination parameters of seeds collected in July in two different harvest years. (A) July 2022, and (B) July 2023. The colour gradient represents values from negative (red) to positive (blue), with lower values represented by darker red and higher values by darker blue. Data were normalized using z-scores to standardize variables. The full names of the species are given in Table 1.

Species collected in early October 2022 and late September 2023, such as *C. jacea* (highest GC and fastest T. Index) and *A. millefolium* (highest DS proportion), showed consistent germination performance in both years. However, changes in environmental conditions resulted in a shift of the two species with the highest germination efficiency in 2022 (*A. angulosum* and *K. dubia*) and 2023 (*H. perforatum* and *S. officinalis*) (Fig. 7A). Higher temperatures, along with lower rainfall in September 2023, led to a decrease in seed germination capacity (GC) and a decrease in germination velocity in *K. dubia* resulting in this species being grouped with *G. boreale* and *G. pratense*, and being among the species with the higher share of FUS and longer t_{50} in both clustering years (Fig. 7B).

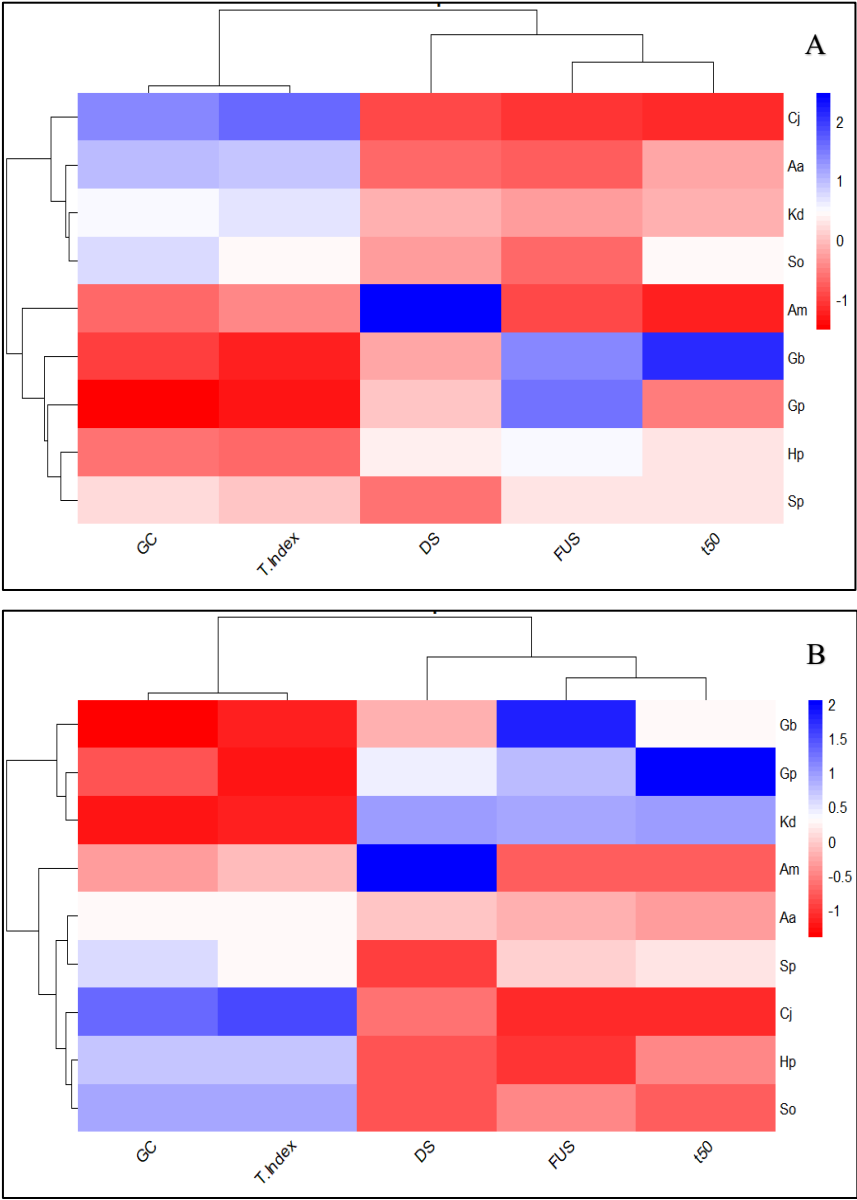


Figure 7. Heatmaps of the analysed germination parameters of seeds collected in September/October in two different harvest years. (A) October 2022, and (B) September 2023. The colour gradient represents values from negative (red) to positive (blue), with lower values represented by darker red and higher values by darker blue. Data were normalized using z-scores to standardize variables. The full names of the species are given in Table 1.

c. Decision trees based on seed maturation periods

Decision tree analysis revealed that the Timson Index was the most important factor explaining GC. For seeds ripening in June and collected in July 2023, the resulting tree consisted of 2 terminal nodes and 1 secondary sub-branch (Fig. 8A). Firstly, the division was made according to the T. index ($< 25\%$ per day). The right branch consists of 13 experimental units with an average T. index of 25% per day or more, which is associated with the highest GC (from 46% to 89%). This number of experimental units is represented by five species, such as *V. angustifolia*, *T. pratensis*, *P. lanceolata*, *E. planum*, and *D. deltoides* (Fig. 8B). Then, the left branch divided into sub-branches with a T. index $< 15\%$ per day (right) resulted in the lowest proportion of GC (on average below 23%) represented in leaf 3 for eight species (*S. flos-cuculi*, *S. autumnalis*, *L. vulgare*, *L. pratensis*, *C. patula*, *B. media*, *A. pratensis*, and *A. elatius*) (Fig. 7B). Next, leaf 4 with moderate germination velocity ($15\% \leq \text{T. Index} < 25\%$) also has eight species.

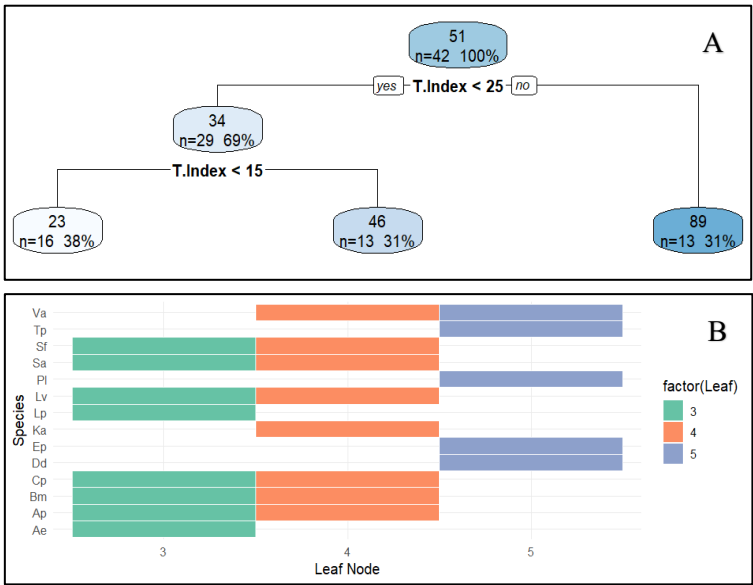


Figure 8. Decision tree for species collected in July 2023. (A) Decision tree split, and (B) Species covering every leaf node. The full names of the species are given in Table 1.

The decision tree of species ripening in September and collected in late September 2023 branches only at two leaf nodes, with exception of *A. angulosum* at both nodes (Fig. 9). The

first node is in leaf 2 and includes five species (*K. dubia*, *G. pratense*, *G. boreale*, *A. millefolium*, and *A. angulosum*) with a T. Index <21% per day, indicating low average GC (Fig. 9A). The second node is also represented by five species (*S. pratensis*, *S. officinalis*, *H. perforatum*, *C. jacea* and *A. angulosum*) with a T. Index $\geq$ 21% per day, reflecting high mean GC (between 27 and 75% and above 75%) (Fig. 9B).

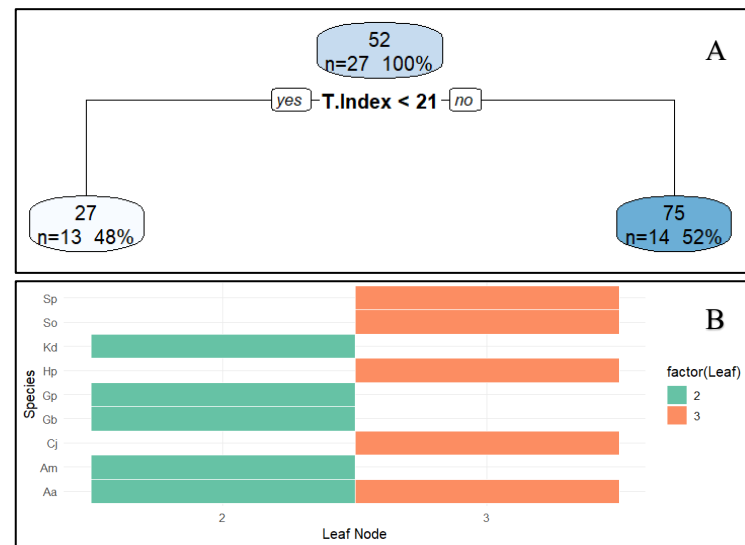


Figure 9. Decision tree for species collected in late September 2023. (A) Decision tree split, and (B) Species covering every leaf node. The full names of the species are given in Table 1.

DISCUSSION

In the present study, we showed that changes in GC, FUS, and DS occurred in different species within similar families, such as the three grasses studied (*A. elatius*, *A. pratensis*, and *B. media*), indicating the influence of weather conditions during seed maturation and harvest. Our next finding regarding the influence of seasonal variation observed in mother plants of perennial grasses was also presented by Bhatt et al. (2018). In this study, low temperatures during seed ripening induced ABA (Absciscic Acid) activation, which could activate the dormancy stage of seeds under unfavourable conditions. Despite the maternal effect, the photoperiod regimes used during germination tests also played a key role in inducing and triggering germination signals for plants. Zhao et al. (2021) found that the maternal effect was suppressed by the incompatibility of photoperiod regimes, as seed maturation time had no significant effect on germination percentage or initial germination time in a germination test conducted under a 12-h dark/12-h light cycle.

Several forbs showed variation in seed germination quantity performances depending on the year of collection. It can be assumed that the greater germination variability in forbs observed in the years studied may be due to life cycle characteristics, such as the number of seeds per

plant, seed survival and dormancy, and the proportion of successfully germinated seeds (Dalglish et al. 2010). Thus, some plants with a short-flowering period were more vulnerable to alternating weather conditions for recruitment than plants with a long-flowering period, such as *T. pratensis*, *C. jacea*, *S. officinalis*, and *S. pratensis*. Price et al. (2023) explained that intra-specific variability can be formed by environmental gradients for forbs germination, even with similar temperature requirements, and this variability in response to specific temperatures can be implemented for the sowing period. Thus, shifting the temperature ranges necessary for germination also affects the dormancy level, where some plants may expand or narrow their favourable environmental range depending on the weather the following year (Kildisheva et al., 2019).

The pattern of germination dynamics can be complemented by considering germination time as a cumulative response to temperature changes (Ghaleb et al., 2022). Our study included an assessment of germination time and velocity, because any change in germination time (t_{50}) directly affected the velocity discrepancies (Caser et al., 2024). Some native species of lowland hay meadows, such as *K. arvensis*, *L. pratensis*, and *G. pratense*, showed shortened t_{50} for seeds collected in 2023. The seed filling and ripening processes of the two above-mentioned species, collected in July (*K. arvensis* and *L. pratensis*), appear to reach optimal ripening with only a slight increase in temperature and rainfall. Conversely, a decrease in total rainfall, followed by the highest temperature increase in 2023, from August to September, may stimulate the ripening of *G. pratense* seeds. This phenomenon revealed a mechanism of plasticity related to the germination niche that could be used to guide the restoration and conservation of local species (Price et al. 2023).

For conservation purposes, it is necessary to maintain appropriate climatic conditions; however, optimizing germination potential and probability enables the development of guidelines for sowing endangered species (Price et al., 2023). This approach has been implemented in Slovenian dry meadows, where the protection of local biodiversity through in-situ conservation should focus on plant communities, and not only on individual plants (Bavcon et al., 2024). Especially in the face of climatic issues affecting seed progeny, species with the highest germinability and the shortest germination time, such as *T. pratensis* and *C. jacea*, may be prioritized in the creation of plant structures enriched with local grasses (*A. elatius*, *A. pratensis*, and *B. media*) to a greater extent to cover the empty spaces in the soil, thus providing an ecological niche (Carta et al. 2022). In the case of legume species, the use of native species (*L. pratensis*) may have conservation value compared to ruderal species (*V. angustifolia*) (Rantala-Sykes and Campbell, 2019). Scotton (2016) assessed the species ratio pattern among

dominant grass species, followed by legumes and forbs, as a key factor in maintaining the sustainability of protected Italian *Arrhenatherion* meadows after 5 years of restoration.

Toma et al. (2023) explained that conservation can guide restoration goals in defining desirable reference habitats. In European semi-natural grasslands, high rates of biodiversity loss due to land-use change and climate change have caused existing species to aggregate in fragmented and isolated patches, thereby reducing the population of reference habitats (Picó and van Groenandel, 2007). One possible approach to enabling local species to revegetate is to reduce pathways between fragmented populations by sowing seeds or introducing new seedlings. Therefore, the focus should be on local species that can germinate at a high percentage and in a short time, as shown in the decision tree analysis. Both the GC and the Timson Index can be used as basic criteria for selecting the species studied. Species with such abilities can occupy free spaces and avoid undesirable plants (Barr et al. 2017).

Several issues should be considered regarding seed quality (Ontivero et al., 2024). Although decision trees showed that some species were positioned on two branches, selection cannot be based solely on germination expression from a single experimental unit, but on all replicates. This can avoid the abnormal pattern of germination dynamics within seed mixtures. However, this phenomenon existed in several characteristic species of *Arrhenatherion* meadows (*L. vulgare*, *C. patula*, *B. media*, *A. pratensis*, and *A. angulosum*). Additionally, a native legume such as *L. pratensis* exhibits low germination performance; however, this species is essential for fixing nitrogen and enriching the soil. Such information can help experts determine when good seed quality can be produced, which species require more than others, and which ecosystem services can be expected to be present following the sowing of target species (Delicour et al. 2023). These conservation-oriented restoration approaches are useful for conserving entire habitats and ensuring the sustainability of ecosystem services, rather than relying solely on enrichment-based restoration (Volis, 2019).

The migration of species from the heatmap plots, the unstable germination efficiency over the two years studied, and the significant variation in germinability, even within one species, can be attributed to the adaptive mechanism of phenotypic plasticity in response to weather changes (Veselá et al. 2021). Moreover, the availability of seeds of native species of appropriate quality can also be achieved through ex-situ conservation, which maintains their stable germinability and rooting capacity and provides a larger number of offspring for large-scale restoration (Shen et al. 2016). Additionally, future studies on germination dynamics should consider other factors, such as seed storage, seed viability, and variation in photoperiod regimes, to better understand the germination processes of local native species (Kolb et al.

2016). In this way, such future research can ensure the availability of high-quality seed sources in sufficient quantities for long-term conservation and restoration.

CONCLUSIONS

Weather shifts during seed ripening determine the seed germination efficiency. Seeds of *A. elatius*, *C. patula*, and *L. vulgare* (collected in July) and *A. angulosum*, *G. boreale*, and *K. dubia* (collected in late September/early October) responded most strongly to these changes. Wetter weather conditions during seed ripening contribute to an increase in the FUS and a decrease in the DS, as well as to an extension of the t_{50} period and a decrease in the Timson Index for most species. Meanwhile, warmer and drier weather causes a decrease in the FUS index, an increase in DS, and an extension of the t_{50} period, but has a smaller effect on the Timson Index (only 4 of 23 species: *A. angulosum*, *G. boreale*, *G. pratense*, and *K. dubia* had a decrease in this index). Species with stable quantity seed germination (GC, FUS, and DS) and quality traits (t_{50} and Timson Index) in both study years were *T. pratensis*, *D. deltooides*, and *P. lanceolata* (collected in July) and *C. jacea* (collected in September/October). These species can be recommended for rapid revegetation and conservation of local, native *Arrhenatherion* meadow species. Furthermore, it was indicated that the Timson Index can serve as a basic parameter for assessing the germination process, explaining seed germination efficiency, and facilitating the selection of introduced species. For conservation-oriented restoration, species with stable values of both parameters (GC and Timson Index) can be prioritized for inclusion in seed mixtures, which are then supplemented with local native species that provide specific ecosystem services.

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