

Physical Properties and Germination Capacity of Black Alder Seeds: Informing Reforestation Efforts for a Threatened Population near Lake Prespa, North Macedonia

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ABSTRACT

Widely spread across Europe, black alder (*Alnus glutinosa* (L.) Gaertn.) is a keystone species in riparian ecosystems. In North Macedonia, the black alder population present in the proximity of Lake Prespa is under severe threat due to the combined impact of anthropogenic factors, climate change, and the age and state of the mature trees. Recent reforestation activities where locally produced seedlings were planted have been conducted; however, significant knowledge gaps regarding the species biology and current state of the reproductive material may decrease the output, i.e., seedling survival. Therefore, in the present study, we analyse the physical and physiological properties of seed lots collected in 2023 and 2024. Furthermore, we also analyse the results from five pre-sowing treatments (cold stratification, ethanol treatment, hydrogen peroxide treatment, water soak, and hydrothermal treatment) selected based on the *A. glutinosa* seed properties. The results indicate a lower viability in the seed lot from 2023, which could be due to the longer storage as well as a potentially low-seed year. The lower viability is reflected in the lower thousand seeds weight (1.165 g in 2023, 1.725 g in 2024), lower germination capacity (20.7% in 2023, 33.4% in 2024), lower germination energy (19.9% in 2023, 31.9% in 2024), and lower viability (18.5% of healthy seeds in 2023, 36.24% in 2024). The comparison between the pre-sowing treatments showed cold stratification as superior to all other treatments, for the seeds lots from both years. Furthermore, with ethanol treatment and water soak, no seeds germinated, indicating a deep dormancy of black alder seeds that needs to be overcome by more intensive physical and/or chemical stimulation. To the best of our knowledge, this is the first study conducted on black alder seeds' germination capacity in North Macedonia. As such, it provides valuable insight into the overall state of the reproductive material as well as practical data on the pre-sowing treatments' effect. With conservation in mind, future research analysing the results of direct sowing as compared to planting, as well as the improvement of nursery production with higher seeding rates (due to the natural low viability of black alder seeds) and the implementation of arbuscular ectomycorrhiza could be of interest.

Keywords: riparian; forest; nursery; plant production; seed characteristics; reforestation; germination

INTRODUCTION

The combined effects of climate change and land-use intensification pose a severe threat for numerous tree species worldwide (Iralu et al. 2019). Reforestation (or revegetation) is a commonly used active restoration

approach that relies on direct seeding on field or planting nursery-grown plant material (saplings) of the target species (Greet et al. 2020). Both methods have positive and negative sides. Direct seeding is less costly but constricted by seed predation, germination, and early seedling survival. On the other hand, while it surpasses these obstacles, the

planting of saplings also requires more inputs in terms of facilities, labour, and management (Moore et al. 2011, Grossnickle and Ivetić 2017, Willoughby et al. 2019). Since seeds are required for the propagation of most tree species, seed germination studies can be an important tool for species conservation, providing insight into the species' plasticity *in situ* and the requirements for germplasm conservation *ex situ* (Iralu et al. 2019). A combined effect of numerous factors conditions the germination outcome, but most relevant are seed coat properties and general morphological seed parameters as internal factors, and temperature, humidity, and water availability as external factors (Kumar et al. 2024). Thus, pre-sowing (or germination) treatments, during which the seed coat can be impacted and ecological factors manipulated, have an important role for the germination success (Chowdhury et al. 2024). Treatment selection is based on the species biology, and commonly involves soaking, stratification, scarification, manipulation of humidity, light, temperature and growing media, and application of plant growth regulators, i.e., plant hormones such as abscisic acid, gibberellins, auxins, etc. (Iralu et al. 2019). Germination success is also impacted by the genetic predispositions and the continuous environmental conditions that preceded the seed production period which need to be considered, especially during the mother plant selection and seed collection phase.

Commonly present in riparian ecosystems, black alder (*Alnus glutinosa* (L.) Gaertn.) is a widespread tree species across Europe (Verbylaitė et al. 2023). It has been found to be of particular value since it can aid water purification from excessive nutrient concentration, provide soil stabilization and erosion control as a pioneer species, has a nitrogen-fixing ability, and can serve as food sources for wildlife during the winter months (Peterjohn and Correll 1984, Mingeot et al. 2016, Willoughby et al. 2019, Sanglyne et al. 2021). The seeds of such riparian plant species are commonly dispersed via anemochory (by wind), hydrochory (by water), and zoochory (by animals) (Fraaije et al. 2017). Since the dispersal mechanisms are crucial for the species survival strategy, and as such have co-evolved with the preferred environmental conditions of the species, they have also impacted the morphophysiological seed characteristics (Levin and Muller-Landau 2000). Please change this sentence to:

In the case of *A. glutinosa*, the seeds -actually winged fruits or achenes containing a single seed without endosperm and surrounded by a pericarp (de Atrip et al. 2007) - are relatively small (2 - 3.5 mm), with low seed specific weight and high buoyancy due to the cork-like seed coat and a waxy surface. Seed production begins when the individuals are 20–30 years old, and every two to three years high seed production occurs, although a certain percentage of viable seeds can be present even in the low seeding years (Göktürk and Güner 2024). Black alder seeds exhibit an orthodox storage behaviour, meaning that when stored in proper conditions (low moisture content and low temperature), they can maintain their viability (Koutsovoulou et al. 2025). Storage conditions and duration can impact germination, and in the case of black alder, studies have shown that seed storage in proper conditions

enables the conservation of the seed quality (Chmielarz 2010, Tylkowski 2014, Koutsovoulou et al. 2025)

In North Macedonia, in the Prespa region located in the south-west part of the country, *A. glutinosa* dominates several types of alluvial riparian forests, i.e., *Alno-Padion*, *Alnion incanae*, and *Salicion albae* (Fotiadis et al. 2018). However, these stands, fragmented and consisting mainly of older individuals, are further threatened by the expansion of orchards in the area, the increasing population of the invasive alien species *Amorpha fruticosa* L., and climate change (Fotiadis et al. 2018). Since this is a priority habitat type (Council Directive 92/43/EEC 1992, Fotiadis et al. 2018), attempts for its conservation also include reforestation efforts since the aforementioned threats have also diminished the natural regeneration potential.

Since an important aspect of species conservation is identifying and providing optimal conditions for seed and seedling survival and establishment (Ehardt-Kistenmacher et al. 2019), practical knowledge regarding seed quality and germination is extremely relevant for improving plant production and the success of the seedlings after their outplanting (Chowdhury et al. 2024). With species such as *A. glutinosa*, whose seeds undergo natural cold stratification in the soil during the winter months, this could be of even higher relevance considering the climate change extremes during which temperatures might fluctuate differently (Twardosz et al. 2021). Therefore, the present study aimed to compare the seed viability and germination capacity of two consecutive sampling years and test how five different pre-sowing treatments impact seeds from *A. glutinosa*. We hypothesized that:

1. the seed quality, reflected in the physical and physiological seed properties will not differ between the seeds collected in 2023 and the seeds collected in 2024;
2. cold stratification, as a pre-sowing treatment, would be most appropriate for black alder seeds.

MATERIALS AND METHODS

Study Area and Seed Collection

The study was conducted in the Prespa region, in the south-west part of North Macedonia, characterized by the presence of Lake Prespa (Figure 1). The infructescences from *A. glutinosa* (cone-like strobiles) were manually gathered from five known mother trees which had been selected due to desirable phenotypic characteristics: overall tree vigour, tree health, vital branches with leaves, relatively rich crowns, absence of mechanical damage, and observed reproductive capacity as indicated by strobile formation. Notably, they were all located in the proximity to rivers. The infructescences were then processed by manual pressing and sieving until the seeds (samaras) were extracted. Both the seeds gathered in October 2023 and October 2024 were conserved in paper bags at 4°C and estimated relative humidity of around 20%, until the seed tests were performed in January 2025. A total of 0.5 kg and 0.5 kg of seeds were gathered in 2023 and 2024, respectively.



Figure 1. Study area in Prespa (North Macedonia) where the cones and seeds from black alder (*Alnus glutinosa* (L.) Gaertn.) were collected (Al. gl. 1–5).

Seed Properties Analysis

The two seed lots, collected in 2023 and 2024, were processed in the same manner but analysed separately for the purpose of the study. From each seed lot, a manually obtained primary sample, which served as a submitted sample, was randomly obtained, in the quantity of 10.99 g (2023) and 12.04 g (2024). In the laboratory, these submitted samples were used as working samples since their weight corresponded with the minimal submitted sample rules indicated by ISTA (ISTA 2025). From each working sample, by randomly taking ten separate scoops with continuous mixing in between, smaller working samples were formed for further analysis. The samples were weighed and cleaned by sieves and the manual removal of non-seed material. This material was first used to measure seed purity (SP) as a physical property used to assess seed quality by comparison of seed weight before and after cleaning calculated as:

$$SP [\%] = \frac{W_{ps}}{TW} \cdot 100 \quad (1)$$

where, W_{ps} is the weight of the cleaned sample, and TW is the total weight of the working sample (Rajendra Prasad 2023, ISTA 2026a).

Then, from these samples, 24×100 smaller samples were separated and weighted for both sampling years (2023 and 2024). Each of them was weighted and used to measure the thousand seed weight (TSW) as a physical property correlated with germination vigour and field performance, and calculated as:

$$TSW [g] = \left(\frac{X_1 + X_2 + \dots + X_n}{n} \right) \cdot 10 \quad (2)$$

where X_1 , X_2 , and X_n indicate the individual weight of working samples of 100 seeds, and n is the number of working samples used (ISTA 2026b).

Regarding the physiological seed properties, we calculated the standard germination capacity (GC), germination energy (GE), and practical seed value (PCV). GC is used for measuring the maximum germination capacity and it is executed by placing four samples of 100 seeds under optimal conditions after exposure to pre-germination treatments. In the present study, this was achieved by placing the seeds on constantly moistened gauze with an adapted growing tray, and maintaining them in constant conditions at room temperature ($22^\circ\text{C} \pm 2^\circ\text{C}$) and high humidity (80%–90%) (Qi et al. 2019). All seeds were observed every 2–3 days, over the period of 28 days, and, if present, germinated seeds were counted and removed from the germination tray. The GC was calculated as:

$$GC [\%] = \frac{N_{gs}}{TN_s} \cdot 100 \quad (3)$$

where N_{gs} is the number of germinated seeds, and TN_s is the total number of tested seeds (Rajendra Prasad 2023).

GE was calculated to evaluate seed vigour, i.e., the speed and uniformity of germination during the first seven days (after transferring to the germination tray) calculated as:

$$GE [\%] = \frac{N_e}{TN_s} \cdot 100 \quad (4)$$

where N_e is the number of germinated seeds in the first 7 days, and TN_s is the total number of tested seeds (Si et al. 2018).

PCV is a derived metric calculated based on seed purity and standard germination capacity used to determine the actual planting value of a seed lot calculated as:

$$PCV [\%] = \frac{SP \cdot GC}{100} \quad (5)$$

where SP is seed purity, and GC is germination capacity.

Furthermore, a seed viability test (VT) was performed to evaluate seed viability and vigour, and to examine the internal structures (embryo, endosperm, cotyledons) without full germination. In the case of *A. glutinosa*, an adapted VT was performed by manually cutting 400 seeds from each sampling year, longitudinally, and noting the state of the seeds (Frischie et al. 2020). The cut seeds were classified in one of the four categories – healthy seeds, empty seeds, rotten seeds, and suspicious seeds. After cutting, the VT was calculated as:

$$VT [\%] = \frac{N_{hs}}{T_s} \cdot 100 \quad (6)$$

where N_{hs} is the number of healthy seeds, and T_s is the total number of cut seeds.

Pre-sowing Treatments

Based on the literature revision and the known seed properties of *A. glutinosa*, five pre-sowing seed treatments

were selected for the purpose of the experiment. A total of 400 seeds (4×100) were used for each of the treatments, as described in detail in Table 1.

Statistical Analysis

For the physical properties (SP and TSW) only descriptive analysis of the relative differences were performed, since single measurements were obtained per year. For the physiological properties (GC, GE, and PCV), the treatment effects were assessed with a two-way ANOVA with interaction terms (Treatment \times Year) for each response variable. When significant effects were detected, Tukey's HSD tests for pairwise comparisons was used. For GE, the data contained zero-inflated values for treatments T3 and T5; therefore, a Kruskal-Wallis non-parametric test was applied instead of ANOVA. For VT, the percentage of each seed category (healthy, empty, rotten, and suspicious) was calculated relative to the total number of cut seeds per year. To assess the seed viability distribution between the two

Table 1. Applied pre-sowing treatments for black alder seeds (*Alnus glutinosa* (L.) Gaertn.).

Treatment code	Treatment name	Treatment description
T1	Cold stratification	The cold stratification treatment for the present study was developed considering literature revision and previous studies (de Atrip and O'Reilly 2007, O'Reilly and de Atrip 2007, de Atrip et al. 2007, Gosling et al. 2009). In detail, the cold stratification was done by placing the seeds in plastic containers with aeration holes, in sterilized moist sand, with no standing water, at 4°C and relative humidity of 100% (in the refrigerator). This aimed to mimic the natural winter conditions and serves as preparation for the spring sowing. The cold stratification lasted for 49 days. Afterwards, the seeds were transferred to the germination tray. Considering it is the most used pre-sowing treatment, in the present study it was used as the control.
T2	Ethanol (C ₂ H ₅ OH) treatment	An adapted treatment based on literature revision included soaking the seeds in 35% ethanol solution, in plastic containers covered with aluminium foil for 24 hours, at room temperature (20–25°C), since ethanol can help in removing or softening the waxy cover of the seed that might be a germination inhibitor (Vishwanath et al. 2013, Shahin et al. 2015, Mensah and Ekeke 2016). Afterwards, they were transferred to the germination tray.
T3	Hydrogen peroxide (H ₂ O ₂) treatment	Adapted method of soaking the seeds in 1% hydrogen peroxide for seven days, at room temperature (20–25°C), in the dark (Ivetić 2002). The method includes three solution changes: i) the seeds are soaked in hydrogen peroxide for 24 hours; ii) the hydrogen peroxide solution is replaced with a freshly prepared batch, and the seeds are soaked for 72 hours; iii) the hydrogen peroxide solution is replaced with a freshly prepared batch, and the seeds are soaked for another 72 hours. Afterwards, they were transferred to the germination tray.
T4	Water soak	During soaking the seeds in water, at room temperature, the seeds swell, which eases the germination (McVean 1955). In the present study, the seeds were soaked in water at room temperature for 35 days, and the water was changed every two days. The prolonged period of hydration was chosen due to the waxy, impermeable seed coat of black alder seeds, and the principle that prolonged hydration may promote greater dormancy release (Gallagher et al. 2004, Bolingue et al. 2010). Afterwards, the seeds were transferred to the germination tray. If seeds germinated during the treatments, they were counted and removed.
T5	Hydrothermal treatment	The hydrothermal treatment, i.e., soaking the seeds in boiling water, could soften the seed coat, and accelerate germination (Alvarado and Bradford 2005, O'Reilly and de Atrip 2007, Burrows et al. 2009). In the present experiment, the seeds were exposed to boiling water (100°C) for 10 seconds, and then left at room temperature water for 24 hours. Afterwards, they were transferred to the germination tray.

years, a Fisher’s exact test was applied, and a two-sample proportion test was used to compare the prevalence of healthy seeds between the years. Statistical significance was assessed at $p < 0.05$. All analyses were conducted in R version 4.3.0 (R Core Team 2021).

RESULTS

The studied samples from both sampling years corresponded with the indicated criteria in the International Seed Testing Association (ISTA) guidelines for *A. glutinosa*, i.e., the maximum weight of lot (in kg) was 1000 kg and the minimum submitted sample (in g) was 8 g (ISTA 2025).

Physical Seed Properties

The results from the physical seed properties indicate an overall increase as all parameters exhibited higher values in the second sampling year. Both SP and TSW were higher in 2024 (Table 2, Figure 2a, 2b).

Physiological Seed Properties

The analysis excluded the seeds subjected to the ethanol treatment (T2) and the water soak treatment (T4). In the case of T2, the seeds from both collecting years were excluded from the analysis since no germination took place. In the case of T4, for samples from 2023, 19

seeds germinated during the water soaking stage, while none germinated once transferred to the germination tray; and for samples from 2024, no seeds germinated during the water soaking stage, and only one seed germination on the germination tray. For the other three treatments (T1, T3, and T5), the two-way ANOVA revealed highly significant treatment effects on all measured parameters ($p < 0.001$) (Table 3). Regarding GC, T1 resulted in superior performance (27.1% mean germination) compared to T5 (9.5%) and T3 (1.8%), with all pairwise differences being statistically significant (Tukey’s HSD, $p < 0.05$). This pattern was further observed in the GE comparison, as T1 exhibited substantial early germination (19.9% in 2023; 31.9% in 2024) while T3 and T5 had complete germination absence at the 7-day evaluation point. Consequently, PCV mirrored these trends, with T1 producing significantly higher values (12.4% in 2023, 24.7% in 2024) than other two treatments ($p < 0.001$) (Table 3). A significant Treatment \times Year interaction indicated that treatment effects varied between years. Notably, with the samples from 2024, T1 showed a germination increase of 12.7% and PCV by 12.3%, compared to 2023 values, while T3 and T5 remained consistently low for both sampling years. In summary, the most substantial treatment differences were observed in the 2024 PCV measurements, as T1 (24.7%) exceeded T3 (0.9%) by 23.8 percentage points, demonstrating the combined impact of treatment efficacy and seed purity on overall seed quality (Table 3, Figure 2c, 2d, 2e).

Regarding the VT, the proportion of healthy seeds increased from 18.5% in 2023 to 36.25% in 2024, while the empty seeds decreased from 81.5% to 56.25% (Table 4). No rotten or suspicious seeds were recorded in either year. Fisher’s exact test indicated a significant difference in the distribution of seed viability categories between the two years. The two-sample proportion test, which compared the number of healthy seeds, also showed a significant increase, from 11.44% in 2023 to 24.06% in 2024, with a 95% confidence interval.

Table 2. Physical seed properties of black alder (*Alnus glutinosa* (L.) Gaertn.) seeds from 2023 and 2024.

Year	Purity (%)	TSW (g)
2023	59.51	1.165
2024	74	1.725

Table 3. The effects of seed treatment and sampling year on physiological seed properties of black alder (*Alnus glutinosa* (L.) Gaertn.).

Parameter	Treatment	2023 Mean \pm SD	2024 Mean \pm SD	Statistical significance	Post-hoc comparison
Germination capacity (GC) [%]	T1	20.7 \pm 7.7	33.4 \pm 3.2	< 0.001*	T1 > T5 > T3
	T3	2.2 \pm 1.5	1.3 \pm 1.5		
	T5	11.8 \pm 9.2	7.3 \pm 5.7		
Germination energy (GE) [%]	T1	19.8 \pm 0.9	31.8 \pm 3.0	< 0.001‡	T1 > (T3=T5)
	T3	0.0 \pm 0.0	0.0 \pm 0.0		
	T5	0.0 \pm 0.0	0.0 \pm 0.0		
Practical seed value (PCV) [%]	T1	12.4 \pm 4.6	24.7 \pm 2.4	< 0.001*	T1 > T5 > T3
	T3	1.3 \pm 0.9	0.9 \pm 1.1		
	T5	7.0 \pm 5.5	5.4 \pm 4.2		

*Two-way ANOVA treatment effect ($F_{2,18} = 42.0$ for final germination; $F_{2,18} = 41.2$ for PCV); †Significant Treatment \times Year interaction; ‡Kruskal-Wallis test ($\chi^2 = 18.9$) due to zero-inflation in T3/T5; T1, cold stratification treatment; T3, hydrogen peroxide (H_2O_2) treatment; T5, hydrothermal treatment

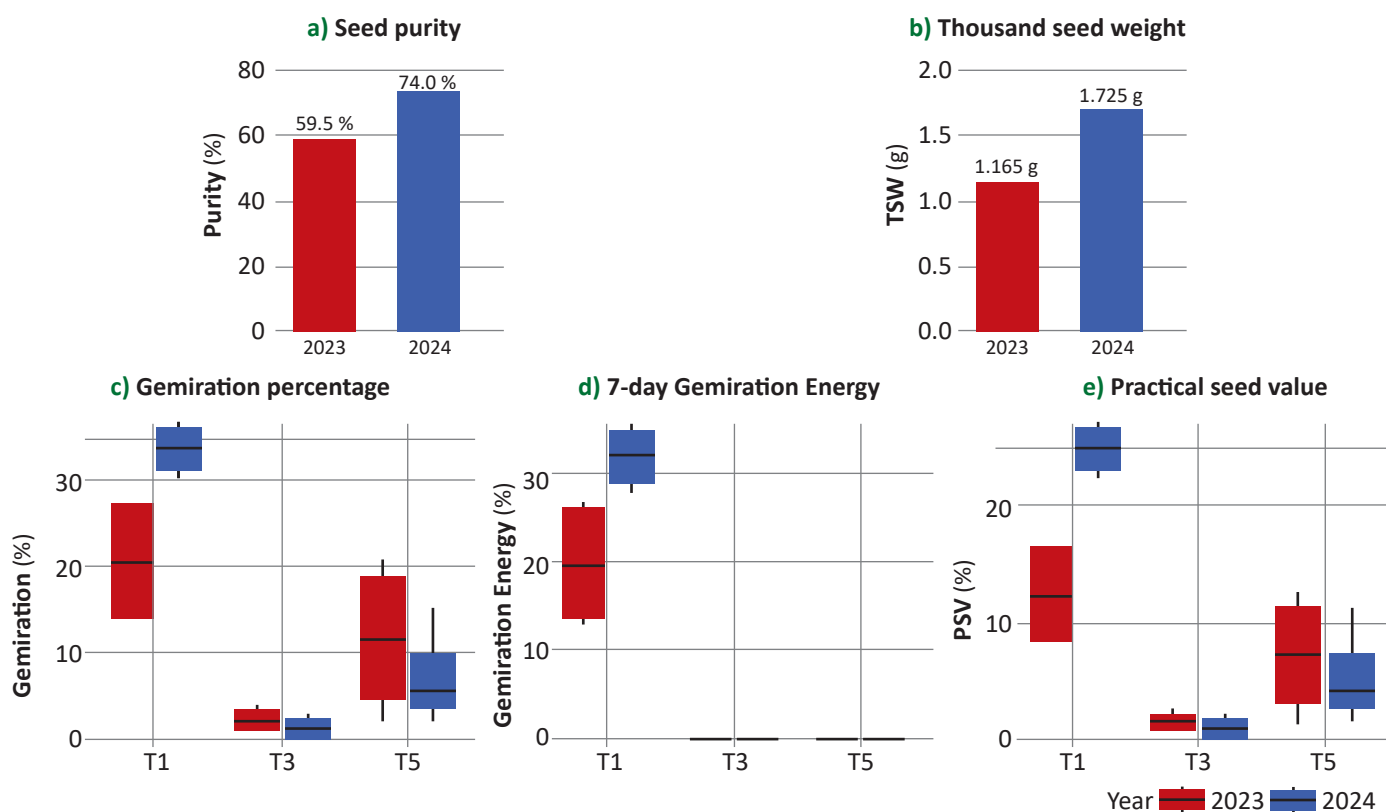


Figure 2. Seed quality analysis of physical and physiological characteristics: **a)** – seed purity; **b)** – thousand seed weight; **c)** – germination percentage; **d)** – 7-day germination energy; **e)** – practical seed value; T1 – cold stratification treatment; T3 – hydrogen peroxide (H₂O₂) treatment; T5 – hydrothermal treatment.

Table 4. The number of healthy, empty, rotten, and suspicious seeds obtained through the seed viability test for black alder (*Alnus glutinosa* (L.) Gaertn.) seeds collected in 2023 and 2024.

Year	Healthy (N)	Rotten (N)	Empty (N)	Suspicious (N)	Total (N)
2023	74	0	326	0	400
2024	145	0	225	0	400

DISCUSSION

The present study provides an investigation of physical characteristics and germination analysis on *A. glutinosa* seeds sampled in two consecutive years (2023 and 2024) in a riparian ecosystem in the Prespa region (in the southwest of North Macedonia) subjected to five different pre-sowing treatments. The results show a difference of the seed properties between the two sampling years, i.e., higher weight and germination rate in the 2024 seed batch. Therefore, we have to reject the first hypothesis as the seeds collected in 2023 show lower quality than the seeds collected in 2024. While it is expected for seeds to lose moisture (i.e., weight) overtime, conservation at lower temperatures has shown to be able to preserve viability in *A. glutinosa* seeds (Hall and Nyong'o 1989, Harrington et al. 2008, Koutsovoulou et al. 2025). However, other studies have shown that *A. glutinosa* seeds stored for two years at 4°C also report a lower germination rate with cold-stratification as a pre-sowing treatment in the

experimental design, or potentially, an insufficient sowing rate (Willoughby et al. 2019). Regardless of the sampling year, we have observed an overall low seed viability and germination capacity, which would further reflect as a low number of seedlings (produced in nursery or obtained by direct seeding in open field). Although species with smaller and lighter seeds usually produce them in higher numbers than those with larger and heavier seeds (Leyer and Pross 2009), the germination rates of *A. glutinosa* are known to be low (de Atrip et al. 2007, de Atrip and O'Reilly 2007, Morales et al. 2012). Self-fertilization can also contribute to seeds with aborted ovules (empty seeds) and such occurrences have been reported in *A. glutinosa* and other alder species (Harrington et al. 2008), which could also explain the overall large number of empty seeds in both sampling years in our case. A high proportion of empty seeds (> 60%) has also been noted in another study in neighbouring Greece (Koutsovoulou et al. 2025). Numerous other factors can contribute to the low seed viability (Sanglyne et al. 2021) and they are not mutually

exclusive, but rather act conjointly. The reduced capacity of seed production could be due to self-fertilization or senescence. Further phenological observations focusing on pollen production and fruit formation need to be conducted for concrete conclusions. However, frequent external disturbances can reduce the seed regeneration capacity in woody plants (Ehardt-Kistenmacher et al. 2019). Climate change, i.e., reduced water availability and prolonged periods of high temperature in crucial parts of the year when the seeds are forming and maturing are a probable scenario, especially since *A. glutinosa* has a higher need for moisture and abrupt precipitation changes are likely to impact seed characteristics (Göktürk and Güner 2024).

The low seed viability is further reflected in the results from the pre-sowing treatments, although they were selected to address seed dormancy, notably present in *A. glutinosa* seeds. Most common inhibitors cause physical dormancy, where the seed coat impermeability can keep the seeds dormant from few months to up to 5 years (Iralu et al. 2019). Indeed, the complete germination absence with ethanol and water soak treatments suggests that dormancy breaking for *A. glutinosa* required physical or chemical stimulation, e.g., complementary treatment combinations such as scarification and soaking in water or specific environmental cues (i.e., temperature and humidity fluctuations) for breaking the dormancy. Seed dormancy and germination characteristics may be due to niche adaptation of a species for securing regeneration (Stromberg et al. 2011, Kanazashi et al. 2015). Considering the ecological niche of riparian species, this is highly likely for *A. glutinosa* and corresponds with the pronounced superiority of the cold stratification across all parameters since the treatment effectively overcame seed dormancy barriers without causing seed damage, likely through enhanced imbibition and the activation of metabolic processes. These results confirm our second hypothesis since the cold stratification showed to be the most effective pre-sowing treatment. The hydrothermal treatment was proven the next most effective after the cold stratification, although not as significantly more as the hydrogen treatment. The hydrothermal treatment involves exposing the seeds to temperature extremes and the hydrogen treatment involves soaking for an extended period in dark conditions. Due to the seed characteristics, future research could explore if combined treatments could be used to provide both physical and chemical stimulation for breaking the dormancy. Dormant seeds can cycle between sensitive (latent soft seeds) or insensitive (hard seeds) stages to dormancy breaking treatments (Taylor 2005). Thus, considering that cold stratification requires more time and in order to ensure higher germination percentages of the viable seeds, further studies that combine it with hydrothermal treatment may be of value. As noted in another study, there is a difference if *A. glutinosa* seeds have been sown in the autumn directly in the soil (mimicking the time of natural seed fall and exposure to the period of moist winter chilling and spring emergence when spring temperatures rise sufficiently) or if they have been artificially pre-chilled and sown in the

spring (which notably provides for later emergence and potentially negative impact of dry and hot conditions during April and May) (Willoughby et al. 2019). Since seed types such as *A. glutinosa* are less likely to succumb to predation, autumn sowing might be better but in the case of spring sowing, pre-chilling of at least 20% of the total seed batch is recommended (Willoughby et al. 2019). Indeed, since temperature has been identified as the most crucial factor for germination, seedbeds in nurseries often provide a suboptimal temperature, especially for the spring sowing since temperature is more difficult to manage than moisture (de Atrip et al. 2007).

Although *A. glutinosa* has a wide natural distribution, from the Mediterranean to mid Scandinavia, it is more adapted to moderate and cooler climate with continuous water availability, which might be an issue if the seasonal characteristics change (i.e., warmer autumns, shorter winters, and dried springs and summers) (Gosling et al. 2009, Nave et al. 2021). Furthermore, small population of mature individuals and continuously reduced seed production can be a characteristic of a threatened species (Iralu et al. 2019). Considering this, to ensure the conservation of *A. glutinosa* in the Prespa region, several restoration activities could be attempted. Considering the high benefit from cold stratification, a potential strategy could be direct seeding in the autumn, directly after collection. In this case, the highest risk would be from low temperatures in the spring, but higher seeding rates that follow recommendations from a seed quality analysis could be a potential mitigation strategy. While it has been noted that an optimal germination temperature for *A. glutinosa* and green alder (*Alnus viridis* subsp. *crispa* (Aiton) Turill) is between 22 and 26°C, these results are based on studies conducted in the United Kingdom and northwestern Ontario where the climatic conditions differ (Farmer Jr. et al. 1985, de Atrip et al. 2007). In the Mediterranean climate, germination occurs as soon as proper moisture is available in the soils, which is why climate change may impact the outcome from known pre-sowing treatments more severely due to reduced rainfall and higher temperatures (Luna et al. 2023). Therefore, it would be of value to monitor how the local climatic conditions impact the on-field germination as well as potential adaptations of the nursery practices.

Additional threats to the populations of *A. glutinosa* in Prespa also need to be anticipated based on relevant research outcomes from other countries. For instance, in Central Europe the host-specific pathogen *Phytophthora alni* species complex is widely present and adapted for water survival (Nave et al. 2021). Since many years can pass from the first symptoms onset (bleeding cankers), and anthropogenic activity is an important transmission factor (22), regional studies in Prespa would be of interest. The risk of invasive species in riparian forests should also be considered. In a study in native floodplain forests in Central Spain that have undergone significant degradation, the exotic *Ailanthus altissima* (Mill.) Swingle, *Ulmus pumila* L., and *Robinia pseudoacacia* L. have exhibited high germination rates, long-lasting seed banks and the capacity for successful germination under heterogeneous

conditions that suppress the high proportion of empty seeds and low germinability in the native *Ulmus minor* Mill. and *Fraxinus angustifolia* Vahl (Cabra-Rivas and Castro-Díez 2016). Considering the invasive species *Amorpha fruticosa* has been noted in the study region (Fotiadis et al. 2018), monitoring of its expansion and proper measures for both *in situ* and *ex situ* conservation of *A. glutinosa* is needed. In the nurseries, cold stratification seems to be the most suitable pre-sowing treatment, and it should be adopted as a regular practice. However, the seeding rate needs to be adapted based on the results from germination rates, as we observe a variability between the two sampling years. Some alternative treatments have been proven effective, e.g., priming seeds of Caucasian alder (*Alnus subcordata* C.A.Mey.) with multi-walled carbon nanotubes which improved the germination under drought stress (Rahimi et al. 2016), or the use of symbiotic relationships between *A. glutinosa* roots with arbuscular ectomycorrhiza and nitrogen fixing bacteria from the genus *Frankia* (Wheeler et al. 1991, Ehardt-Kistenmacher et al. 2019). However, such practices are often costly and require a specific infrastructure, which might be a major obstacle. Further nursery and field-based studies exploring this interaction might also support the seeding establishment.

CONCLUSIONS

Riparian ecosystems provide numerous benefits and, as such, are of a particular conservation interest. In the Prespa region, in south-west North Macedonia, black alder, one of the main species in the riparian ecosystem, is under threat due to combined effect of naturally occurring and anthropogenic factors. Reforestation efforts currently rely on seedling production from locally collected seeds. However, no information is available on the state of the mother trees, and thus the seed quality. Furthermore, since black alder seeds require pre-sowing treatment, it is of great practical value to understand how different treatments impact the seeds and subsequently select the most suitable one. The present study addresses both issues, by analysing seeds collected in two consecutive years (2023 and 2024) and a comparative analysis of five pre-sowing treatments, selected based on the black alder seeds' properties. The results show that the seeds collected in 2024 are of higher quality. This can be linked to the negative impact of seed storage on the seed viability. Regarding the pre-sowing treatments, cold stratification has shown to be the most effective pre-sowing treatment,

followed by hydrothermal treatment and hydrogen peroxide treatment, clearly indicating the need for physical and/or chemical stimulation to remove the dormancy. On the contrary, the ethanol treatment and the water soak had no effect on the seed germination. These results emphasize the need for continuous monitoring of the seed quality and quantity of *A. glutinosa* from the region, as well the analysis of the state of the mother trees and their potential diversification during the collection. Notably, the current study is limited due to the relatively small sample size and the results should be carefully considered. However, considering the significant knowledge gap for the black alder, especially in terms of seed quality and processing, it might provide insights and bases for future research. For future conservation efforts, on-field implementation of direct seeding in test plots could be useful to observe how the seeds will behave in natural conditions. At the same time, nursery seedling production must include cold stratification to ensure germination of the viable seeds. According to the conditions and availability, combining cold stratification with hydrothermal treatment and testing the effect of arbuscular ectomycorrhiza and nitrogen fixing bacteria from the genus *Frankia* could also be implemented.

Author Contributions

AD, SP, and DDK conceived and designed the research, SP carried out the field sampling, AD and DDK performed laboratory analysis, AD, OO, and IM prepared the figures, AD processed the data and wrote the draft version of the manuscript, AD, SP, OO, IM and DDK revised and edited the manuscript. AD dealt with the revision process.

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Conflicts of Interest

The authors declare no conflict of interest.

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