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Ecophysiology of seed germination in riparian trees and implications for restoration and conservation

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Abstract

Riparian ecosystems are essential for ensuring linear habitat connectivity and providing thermal refugia for wildlife. However, they are increasingly vulnerable to global change, resulting in widespread degradation. Four riparian trees, specifically *Alnus glutinosa*, *Alnus incana*, *Platanus orientalis*, and *Liquidambar orientalis*, have been designated as case studies for ex situ conservation due to their ecological importance and vulnerability to habitat degradation. Seeds of the four species were collected in Greece. Germination experiments were conducted at seven constant and three daily alternating temperature regimes, under light or dark conditions. In *A. glutinosa*, *A. incana*, and *L. orientalis*, germination was also tested following a cold stratification pretreatment. For all species, the highest germination was obtained at high temperatures (30°C and 30/20°C). Cold stratification pretreatment expanded the temperature range suitable for germination and increased the germination percentage under unfavorable temperatures. Light promoted germination of untreated seeds across all species. Secondary physiological dormancy by adverse temperatures (thermodormancy) or darkness (skotodormancy) was not imposed. The seeds of all four riparian trees exhibit orthodox storage behavior, as there is no differentiation in germination (both in terms of final percentage and rate) among (a) untreated seeds, (b) desiccated seeds, and (c) seeds that have been desiccated and stored at −20°C. The germination responses of seeds of the four species to temperature, light, and cold stratification reveal the mechanism that delays germination from autumn/early winter (time of seed dispersal) until spring, when conditions are favorable for seedling establishment.

KEYWORDS

cold stratification, light response, riparian trees, seed germination

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1 | INTRODUCTION

Riparian ecosystems are inherently resilient and play a crucial role in ensuring habitat connectivity and serving as thermal refugia for wildlife, thereby linking aquatic and terrestrial ecosystems (Seavy et al., 2009). These ecosystems, situated at the interface of inland freshwater boundaries, integrate both the physical environment and a diverse array of biological communities, resulting in highly complex and diverse systems. Despite the significance of these critical habitats, and their ecosystem services, riparian zones are increasingly susceptible to global change, leading to widespread degradation. Recognizing the pivotal role of riparian ecosystems, there is a growing consensus on the need for ecological restoration to address multifaceted environmental pressures (Rodríguez-González et al., 2022). The term "riparian forests" denotes the vegetation found within floodplains along rivers and streams, with their developmental and structural patterns intricately influenced by responses to disturbances, soil dynamics, and biological characteristics associated with successional processes (Naiman et al., 1998).

Four riparian trees, specifically *Alnus glutinosa* (L.) Gaertn. (Betulaceae), *Alnus incana* (L.) Moench (Betulaceace), *Platanus orientalis* L. (Platanaceae), and *Liquidambar orientalis* Mill. (Altingiaceae), have been designated as study cases for ex situ conservation in the current study (Ickert-Bond & Wen, 2013; Stevens, 2001).

The common alder (*A. glutinosa*) is a native European tree, distributed from western Siberia to Iran and northwestern Africa (Houston Durrant et al., 2016a; Kajba & Gracan, 2003; POWO, 2024). Known for its tolerance to diverse environments, it is often found near streams, rivers, lakes, or areas with poor soil drainage. The species adapts to a wide temperature range and demonstrates notable frost tolerance. Its optimal growth occurs in continental climates with abundant water availability. *Alnus glutinosa* typically grows below 1000 m a.s.l., although in central European mountains it can extend along watercourses up to 1800 m a.s.l. (Houston Durrant et al., 2016a).

Alnus incana, commonly known as gray alder, is a smaller and relatively short-lived deciduous tree distributed throughout the Northern Hemisphere. Typically thriving in riparian areas, this species exhibits exceptional frost tolerance and can grow up to the tree line in northern European regions. Notably, it replaces the common alder (*A. glutinosa*) at higher elevations in central Europe, demonstrating frost resilience and reaching the northern forest border in Scandinavia and European Russia, with its expansion limited only in areas of permafrost (Houston Durrant et al., 2016b). *Alnus incana* is classified as near threatened (NT) according to the Red Data Book of rare and threatened plants of Greece (Xystrakis et al., 2009).

Alders, as pioneer species, require high light levels for optimal growth and are commonly found on exposed mineral soils. Their ability to fix atmospheric nitrogen facilitates their establishment on geologically young or disturbed sites (Harrington et al., 2008). Additionally, common alder forms forest galleries, contributing to the European Community priority habitat type 91E0*, designated as "Alluvial forests with *Alnus glutinosa* and/or *Fraxinus excelsior* (Alno-Padion, Alnion incanae, Salicion albae)." Both alder species are monoecious and wind pollinated, typically flowering in spring (February–March). Their cones, hereafter called infructescences, have small winged nutlets, hereafter called seeds, arranged in pairs on each bract, which are dispersed in autumn (October–December) (De Atrip et al., 2007; Harrington et al., 2008; Xystrakis et al., 2009).

The natural range of oriental plane (*P. orientalis*) populations extends from the Eastern Mediterranean region, including Calabria and Sicily (Bonner, 2008; Caudullo et al., 2017; POWO, 2024; Strid & Tan, 2002). Its flowering period occurs in spring (March–May), with fruits, hereafter called infructescences, maturing in autumn, and most conical nuts adorned with hairy tufts, hereafter called seeds, are dispersed in September–October (Arampatzis, 1998; Bonner, 2008; Strid & Tan, 2002).

Oriental sweetgum (*L. orientalis*) is a tertiary relict endemic to the eastern Mediterranean (Öztürk et al., 2008) and is found in southwest Turkey and Rhodes Island, Greece, at elevations ranging from 0 to 1000 m a.s.l. (Alan & Kaya, 2003). Its unisexual flowers open in spring (March–April) and its fruits are manifested in woody infructescences enclosing 25–30 (−40) helically arranged capsules. These infructescences ripen in late autumn (November–December), and seeds are wind dispersed.

Platanus orientalis and *L. orientalis* both contribute to the European habitat type 92C0 "*Platanus orientalis* and *Liquidambar orientalis* woods (Platanion orientalis)." However, natural stands of oriental plane in Greece face significant challenges from the invasive fungal pathogen *Ceratocystis platani* (Ocasio-Morales et al., 2007). Urgent conservation measures are needed to address this threat.

The four riparian trees were selected for this study because (a) the *Alnus* species contribute to the priority habitat 91E0* and reach their southernmost limit in Greece, (b) oriental plane trees are experiencing severe die-offs in riparian forests, and (c) *L. orientalis* is a tertiary relict tree endemic to the Eastern Mediterranean region, with a very restricted natural distribution in

TABLE 1 Collection data, seed mass and percentages of sound, infected, and empty seeds for *Alnus glutinosa*, *Alnus incana*, *Liquidambar orientalis*, and *Platanus orientalis* collections ($n = 100$).

Taxon	Collection site	Collection date (year-month)	Coordinates (° N–° E)	Altitude (m a.s.l.)	Seed mass (mg)	Sound (%)	Infected (%)	Empty (%)
Alnus glutinosa (A1)	Rodopi (northern Greece)	2014-11	41.25–24.76	1053	0.75	35	2	63
Alnus glutinosa (A2)	Vistriza (central Greece)	2014-10	38.88–22.16	188	1.59	27	3	70
Alnus incana	Rodopi (northern Greece)	2014-10	41.47–24.15	1090	0.40	28	1	71
Liquidambar orientalis	Rhodes Island (southeastern Greece)	2014-12	36.15–28.00	100	2.42	93	0	7
Platanus orientalis	Taygetos Mountain (southern Greece)	2015-01	37.11–22.20	400	3.22	73	0	27

Europe (Strid, 2024). All four trees play a critical role in riparian zones and significantly contribute to their biodiversity (Aguiar et al., 2013; Dimopoulos et al., 2010, 2013). However, they are notably sensitive to hydrological alterations and are presumably impacted by climate change. The restoration of riparian areas involves implementing management techniques to enhance forest development in these zones, thereby improving instream and riparian habitat conditions (Pollock et al., 2005). Given their high conservation value, the protection and restoration of riparian areas have become global priorities in land management (Boisjolie et al., 2020). Riparian forest planting is proposed as a mitigation measure to reduce water temperatures, benefiting aquatic organisms (Kristensen et al., 2013).

The aim of this study is to examine the seed germination requirements and seed storability of the aforementioned four riparian trees. This work is essential for developing effective conservation management plans and restoration strategies for environmentally sensitive riparian habitats.

2 | MATERIALS AND METHODS

2.1 | Seed collections

Four riparian species were chosen as subjects for the reproductive study, namely *A. glutinosa*, *A. incana*, *L. orientalis*, and *P. orientalis*. Cones/infructescences of the four study species were collected in Greece (Table 1).

Fruits of the four species were air dried in the laboratory for a few weeks to facilitate natural desiccation and opening. Subsequently, seeds were extracted manually or by employing a shaking method in plastic containers to ensure the release of all seeds from the cone or infructescence. In certain cases, sieves and a column blower were utilized to enhance the separation process.

2.2 | Infructescence biometric characteristics

Biometric characteristics of infructescences, specifically diameter (recorded in millimeters) and mass (measured in grams), were assessed for *A. glutinosa* (A2), *L. orientalis*, and *P. orientalis* immediately after collection. These characteristics provide useful information for conservation practitioners to plan seed collections and effectively implement reforestation efforts. The measurements were conducted using an electronic digital Vernier caliper for diameter and an analytical balance for mass. Following the opening process and subsequent measurements, the number of seeds per infructescence and seed mass were determined. The recorded data are presented in Table 2. The infructescences of *A. glutinosa* (A1) and A. incana had already opened prior to the measurement process.

For all seed collections, the seed mass of 100 seeds was recorded, along with the percentage of sound, infected, and empty seeds, as presented in Table 1.

2.3 | Seed germination

Seed germination experiments were initiated a minimum of 2 months and a maximum of 1 year post collection; seeds were maintained in containers at room temperature for that period. Imbibition procedures were conducted in Petri dishes containing filter paper saturated with

TABLE 2 Biometric characteristics of *Alnus glutinosa*, *Liquidambar orientalis*, and *Platanus orientalis* infructescences ($n = 10$). Values are represented as means ± SE.

Taxon	Value	Infructescence mass (g)	Diameter (mm)	Seed mass/ infructescence (g)	Seed mass/ infructescence mass (%)	Seed number/ infructescence
Alnus glutinosa (A2)	Mean	1.0 ± 0.1	11.2 ± 0.6	0.12 ± 0.02	13.1 ± 1.1	73 ± 10
	Min–Max	0.4–1.5	8.1–12.9	0.06–0.24	5.1–47.5	31–124
Liquidambar orientalis	Mean	4.9 ± 0.3	2.6 ± 0.1	0.40 ± 0.04	8.1 ± 0.5	157 ± 15
	Min–Max	2.1–6.5	2.1–3.0	0.13–0.54	6.0–10.6	45–216
Platanus orientalis	Mean	4.4 ± 0.6	3.1 ± 0.1	3.61 ± 0.50	82.7 ± 2.2	849 ± 91
	Min–Max	1.6–7.4	2.5–3.8	1.46–6.89	72.0–93.1	428–1238

distilled water. The seed samples, comprising either 25 or 20 seeds, were determined based on the proportion of empty seeds; for each experiment, five seed samples were used. Notably, in the experiments involving *P. orientalis* conducted in darkness, two samples were utilized, each containing 60/65 seeds. Following the completion of germination experiments, cut tests were conducted, and germination percentages were adjusted to account for viable seeds. In experiments conducted under darkness, imbibition and seed measurements occurred in the presence of green safelight, and Petri dishes were stored in lightproof metal containers.

The germination rate (T_{50}), denoting the time required for 50% of the final germination to be achieved, was calculated following the methodology outlined by Skourti and Thanos (2015).

Germination experiments were systematically conducted under controlled conditions involving seven constant temperatures (5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C) and three alternating temperature regimes (20/10°C, 25/15°C, 30/20°C; 12 h/12 h). The selection of optimal germination temperatures was based on a consideration of final germination percentage and T_{50}. Subsequently, seeds failing to germinate under suboptimal conditions were transferred to optimal conditions to elucidate the potential induction of secondary physiological dormancy imposed by adverse temperatures (thermodormancy).

The impact of light was initially investigated through two distinct conditions: diurnal cycles of white light and darkness (12 h/12 h) and continuous darkness (D). For light-requiring species, seeds that did not germinate in darkness were subsequently transferred to light conditions to investigate the potential induction of secondary physiological dormancy imposed by darkness (skotodormancy). Phytochrome activity in *A. glutinosa* (A1) and *A. incana* was investigated at a temperature of 25°C under five distinct experimental conditions: (a) diurnal cycles of white light and darkness (12 h/12 h), (b) continuous darkness, (c) darkness with a 10-min exposure to red light (R), (d) darkness with a 15-min exposure to far-red light (FR), and (e) darkness with a 10-min exposure to R followed by 15 min of FR. Light treatments commenced 24 h post seed imbibition. Red and FR light pulses were administered within lightproof chambers. Red light was emitted by four red fluorescent bulbs (Philips TL 20 W/15), filtered through a red plexiglass sheet. Far red light was emitted by four white incandescent tubes (Sylvania 60 W), filtered through one red and two blue plexiglass sheets. Additionally, germination under continuous FR was examined in *L. orientalis* and *P. orientalis* at temperatures of 25/15°C, under three distinct experimental conditions: (a) diurnal cycles of white light and darkness (12 h/12 h), (b) continuous darkness, and (c) diurnal cycles of FR and darkness (12 h/12 h). For the FR treatment, white light was filtered through one red and two blue Plexiglas sheets, each 3 mm thick (Röhm GmbH, Germany). Germination was assessed once after 15 days to mitigate any potential influence from green light exposure on germination.

The durations of cold stratification tested were 1 and 2 months for *Alnus* species and *L. orientalis*, depending on seed availability. Following cold stratification, the seeds were transferred to temperatures of 10°C, 15°C, 20°C, and 25°C under light/dark cycles.

2.4 | Seed storage behavior

The evaluation of seed storage behavior of the four species was performed by following the protocol outlined by Hong et al. (1998). Desiccation procedures were conducted in a sealed chamber utilizing silica gel as desiccant, and relative humidity measurements were carried out using a digital hygrometer. Seeds designated for storage at −20°C were placed in hermetically sealed glass containers containing silica gel to maintain desiccation conditions. Following the drying and storage period at

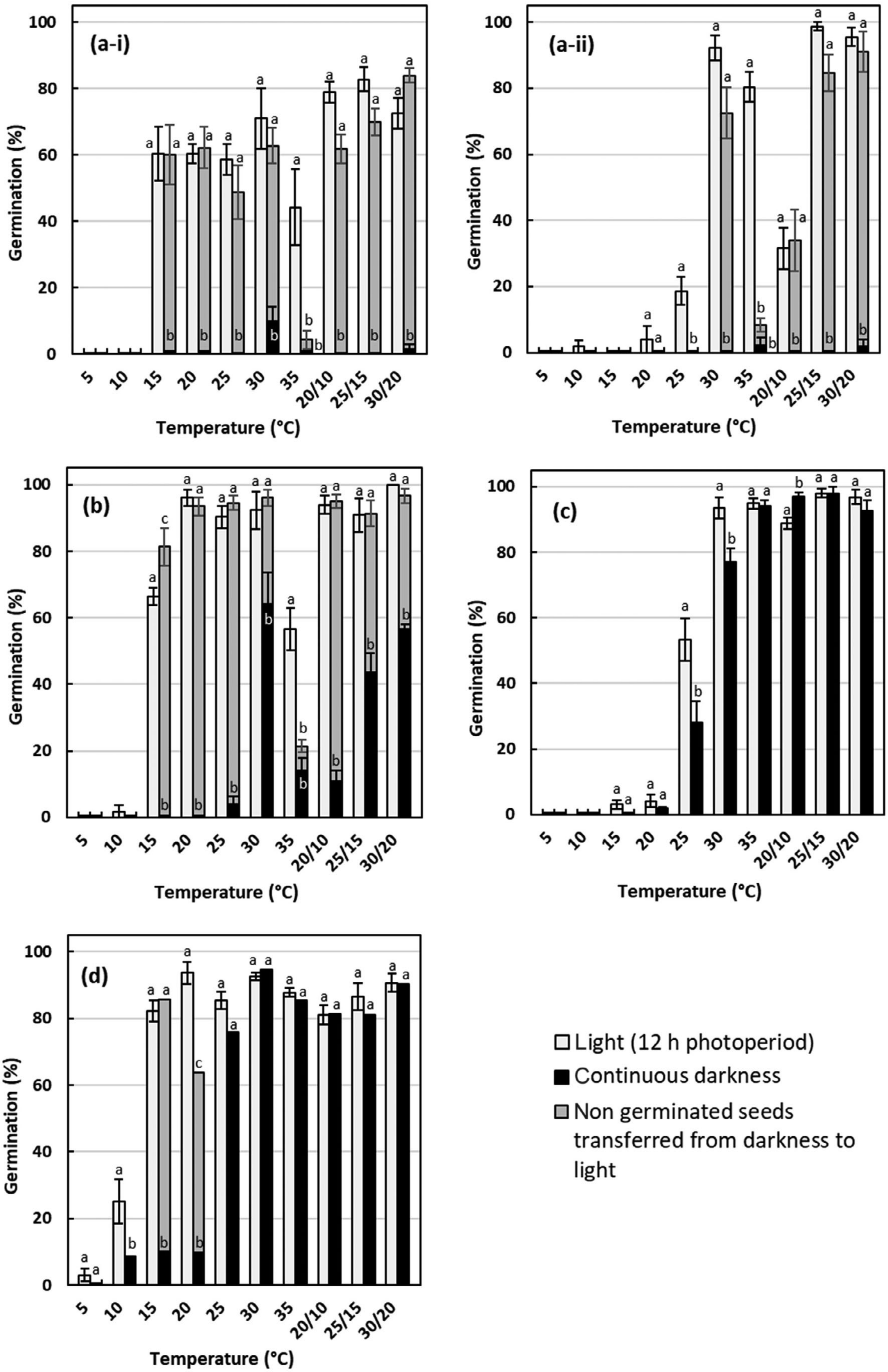

FIGURE 1 Legend on next page.

−20°C, the final germination assessments were performed in light at 30°C, which are the optimal conditions for each species.

2.5 | Statistical analysis

Statistical comparisons among (1) temperatures, (2) light conditions at various temperatures in untreated seeds, (3) durations of cold stratification pretreatment, and (4) storage conditions for each seed lot were conducted using the nonparametric Kruskal–Wallis test, followed by the Dunn test for post hoc analysis. These analyses were carried out using the R statistical software version 4.3.2 (R Development Core Team, 2021).

3 | RESULTS

The contribution of seed mass to the infructescence mass is notably high in *P. orientalis*, reaching approximately 80%. In contrast, it is below 15% for both *L. orientalis* and *A. glutinosa* (A2) (Table 2). In terms of the number of seeds per infructescence, *P. orientalis* also holds the highest count at 849 ± 91, followed by *L. orientalis* with 157 ± 15, and *A. glutinosa* (A2) with 73 ± 10 (Table 2).

Additionally, the two *Alnus* species have the lowest seed mass but a higher proportion of empty seeds, exceeding 60% in contrast to *P. orientalis* and *L. orientalis*, both of which have less than 30% empty seeds (Table 1).

Untreated seeds germinated at ≥15°C for *A. glutinosa* (A1), *A. incana*, and *P. orientalis*; ≥25°C for *L. orientalis*; and ≥30°C for *A. glutinosa* (A2) (Figure 1). However, few or no seeds germinated at lower temperatures (typically below 25%). At very high temperatures ≥35°C, the final germination was above 80% for *A. glutinosa* (A2), *L. orientalis*, and *P. orientalis*, while it was 44%–57% for *A. glutinosa* (A1) and *A. incana*. At alternating temperatures (20/10°C, 25/15°C, and 30/20°C), final germination was above 70%, with the exception of *A. glutinosa* (A2) at 20/10°C. The optimal germination conditions for all species were 30°C and 30/20°C.

The germination rate of untreated seeds was notably high, with T_{50} values ranging between 2 and 12 days in light germination experiments. The quickest germination, as indicated by T_{50} values of 2–3 days, occurred at temperatures exceeding 25°C for all four species.

Thermodormancy induction was investigated for seed collection A1 at 10°C; for A2 at 10°C, 15°C, and 20/10°C; for B at 10°C; for C at 10°C, 15°C, and 20°C; and for D at 10°C. Germination was significantly promoted following transfer to favorable temperatures; therefore, no thermodormancy was imposed.

Light-induced germination of untreated seeds (Figure 1) was noted across all species, occurring at least under certain temperature conditions tested ($p < 0.05$). For *Alnus* species, promotion of seed germination by white light ($p < 0.05$) was consistently observed across all temperature conditions. In *A. glutinosa* (seed lots A1 and A2) and *A. incana*, germination in darkness did not exceed 10% and 64%, respectively. In addition, seed germination of the two *Alnus* species was promoted by R and suppressed by FR, thus showing a typical phytochrome reaction (Table 3). For *L. orientalis* and *P. orientalis*, germination was light indifferent at the majority of temperatures studied, including under FR/D conditions (Table 3). The ungerminated seeds from experiments conducted in darkness exhibited sufficient germination after being transferred from dark to light conditions (Figure 1), indicating that the seeds were not skotodormant ($p < 0.05$), except for 35°C in *Alnus* species and 20°C in *P. orientalis*.

In all four species, cold stratification pretreatment expanded the temperature range suitable for germination and increased germination percentage under unfavorable temperatures ($p < 0.05$). One month of cold stratification was sufficient to promote seed germination at temperatures 10°C–25°C in all species, with the exception of *L. orientalis*, for which 2 months of cold stratification were required to induce germination at 10°C (Figure 2). A reduction in the germination rate was also observed following stratification.

The seeds exhibit orthodox storage behavior, as there is no differentiation in germination (both in terms of final percentage and rate) between untreated seeds and

FIGURE 1 Final germination in untreated seeds of *Alnus glutinosa* (a-i: collection A1 from central Greece; a-ii: collection A2 from northern Greece), *Alnus incana* (b), *Liquidambar orientalis* (c), and *Platanus orientalis* (d), tested over a range of constant (5°C, 10°C, 15°C, 20°C, 25°C, 30°C, and 35°C) and alternating temperatures (20/10°C, 25/15°C, and 30/20°C), in light (12 h photoperiod, white bars) or in continuous darkness (black bars). Nongerminated seeds in continuous darkness were subsequently transferred to light (gray) only in cases where germination in light was significantly higher than in darkness. Experiments were conducted 2–3 months after seed collection, except for those at 30°C, 35°C, and 30/20°C, which were conducted 5–7 months after collection. Different lowercase-letter labels indicate significantly different values between light and dark treatments within each temperature ($p < 0.05$). Vertical lines correspond to ± standard error ($n = 5$, except for *Platanus orientalis* in darkness, where $n = 2$).

TABLE 3 Final seed germination (%) of *Alnus glutinosa* (A1), *Alnus incana*, *Liquidambar orientalis*, and *Platanus orientalis* under different light regimes. L/D, light/darkness (12 h photoperiod); cD, continuous darkness; R, darkness with a 10-min exposure to red light; FR, darkness with a 15-min exposure to far-red light; R&FR, darkness with a 10-min exposure to R followed by 15-min FR; FR/D: far-red light/darkness (12 h photoperiod). Light treatments commenced 24 h post seed imbibition. Values are represented as means ± SE ($n = 5$).

Taxon	L/D	cD	R	FR	R&FR	FR/D
Alnus glutinosa (A1)	76.9 ± 2.0	0.0 ± 0.0	63.4 ± 3.3	0.0 ± 0.0	0.0 ± 0.0	-
Alnus incana	98.6 ± 1.4	13.3 ± 3.8	88.9 ± 4.4	8.3 ± 4.6	5.6 ± 3.5	-
Liquidambar orientalis	92.8 ± 2.6	95.7 ± 2.1	-	-	-	87.4 ± 2.7
Platanus orientalis	87.9 ± 2.7	79.9 ± 3.0	-	-	-	78.6 ± 6.4

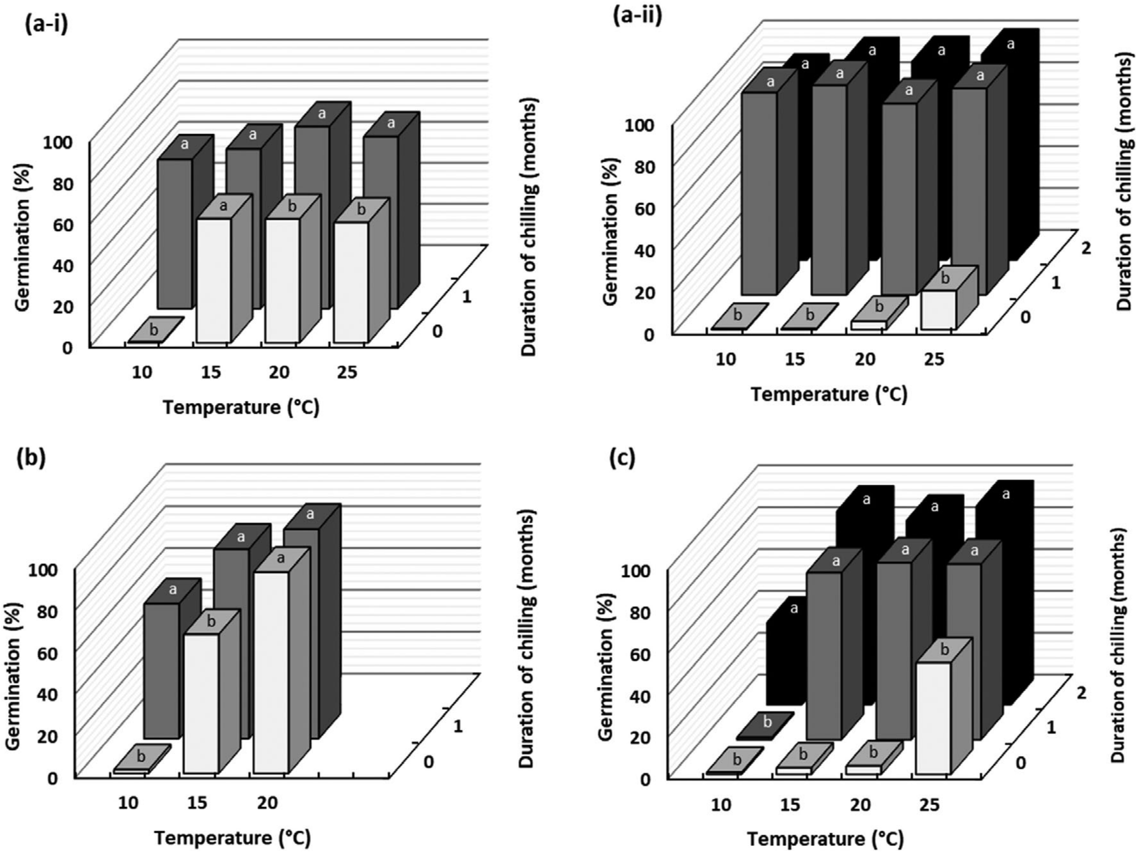

FIGURE 2 Effect of various cold stratification durations (0, 1, and 2 months) on final germination of *Alnus glutinosa* (a-i: collection A1 from central Greece; a-ii: collection A2 from northern Greece), *Alnus incana* (b), and *Liquidambar orientalis* (c) seeds at 10°C, 15°C, 20°C, and 25°C, under white light (12 h photoperiod). Different lowercase-letter labels indicate statistically significant differences among germination values obtained by different cold stratification durations within each temperature ($p < 0.05$, $n = 5$).

those that had been desiccated or those that had been desiccated and stored at −20°C (Figure 3).

4 | DISCUSSION

Ex situ conservation plays a pivotal role in both current conservation efforts and future restoration actions, enhancing the chances of species survival in the face of environmental challenges. Climate change presents various challenges for these ecosystems, including shifts in precipitation patterns, temperature fluctuations, and alterations in hydrological cycles. In addition, the degradation of these ecosystems globally has emerged as a significant threat to the ecosystem services provided by riparian areas and their vegetation, including genetic resources (Riis et al., 2020). An understanding of germination requirements and storage behavior of riparian species is crucial for the efficacy of ex situ conservation efforts. Furthermore, the practices involved in plant

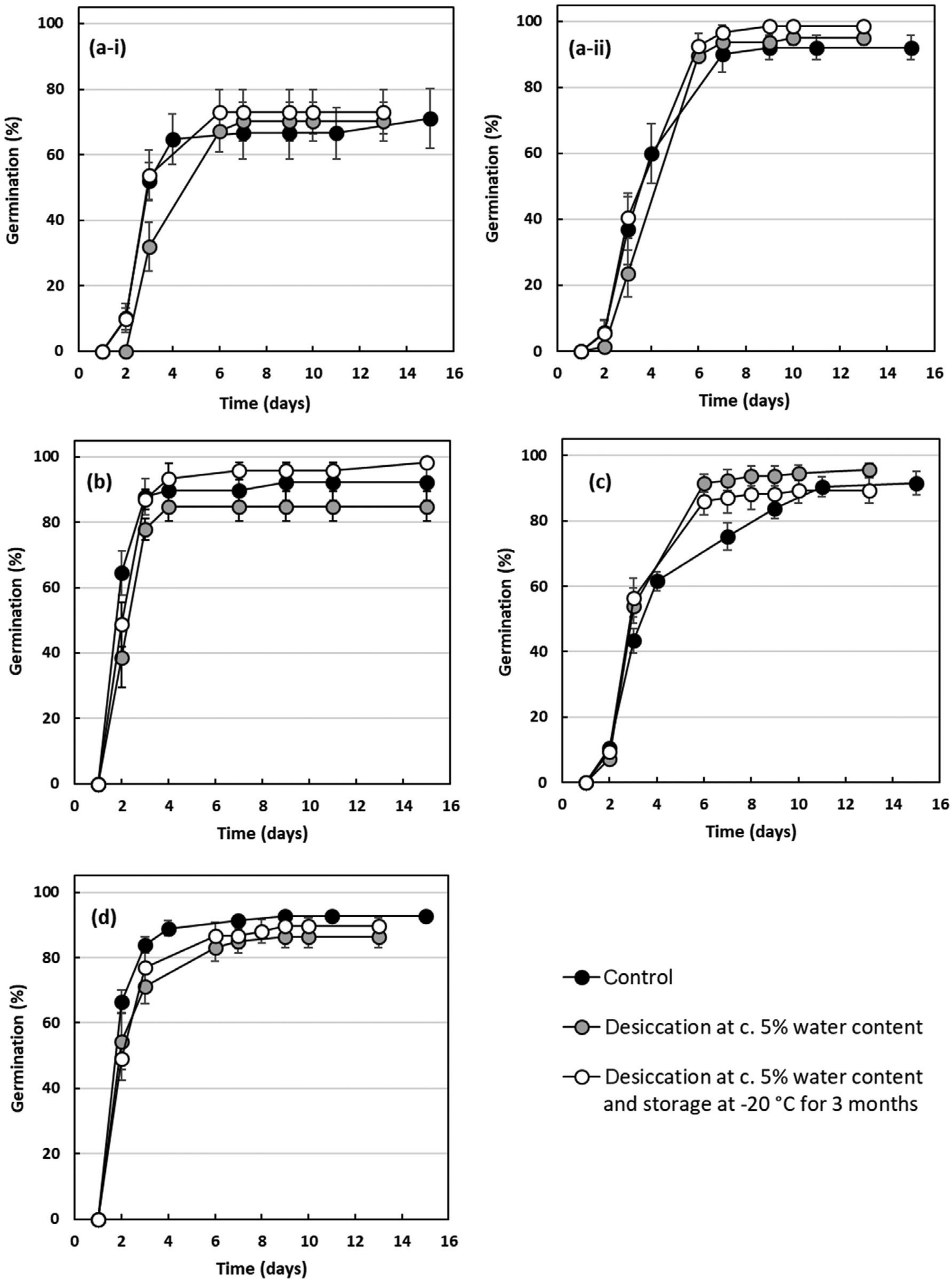

FIGURE 3 Time course of seed germination of *Alnus glutinosa* (a-i: collection A1 from central Greece; a-ii: collection A2 from northern Greece), *Alnus incana* (b), *Liquidambar orientalis* (c), and *Platanus orientalis* (d) at 30°C (12 h light/12 h dark), without any treatment (black circles), after desiccation at c. 5% water content (gray circles) and after desiccation and storage at −20°C for 3 months (white circles) in the NKUA Seed Bank. Experiments were conducted between 8 and 12 months following seed collection. Vertical lines correspond to ±standard error ($n = 5$).

production from seeds can impact the genetic diversity of cultivated plant materials, consequently influencing their potential to adapt to a reintroduction site (Diaz-Martin et al., 2023).

Seeds of the four species of riparian trees exhibited high germination percentages at elevated temperatures, exceeding 15°C, or even 25°C in some cases. Optimal germination conditions are often observed at 30°C or in alternating temperatures of 30/20°C. Seeds showed an increase in the temperature range for germination following a short period of cold stratification (1–2 months). The same germination strategy, that is, optimal germination at warm temperatures and enhancement of germination rate and percentage at suboptimal conditions, has been found in other tree species (e.g., *Abies cephalonica*, Daskalakou et al., 2018).

The findings concerning germination temperatures of the two alder species are consistent with the results of other researchers (De Atrip & O'Reilly, 2005; Gosling et al., 2009; O'Reilly & De Atrip, 2007; Schalin, 1967), who generally recommend high temperatures, sometimes preceded by cold stratification. Technical documents and guidelines often differentiate between fresh and dried seeds concerning the cold stratification requirements (Aránzazu-Prada & Arizpe, 2008; Piotto & Di Noi, 2003). In this study no experiments have been conducted on freshly collected seeds of the four study species. The extension of the temperature range following cold stratification may indicate primary physiological dormancy. For *L. orientalis*, temperatures ranging from 15°C to 25°C are recommended (Öztürk et al., 2008), while a cold moist stratification period (lasting 4–6 weeks) that induces uniform germination is reported for *L. styraciflua* (Aránzazu-Prada & Arizpe, 2008).

While germination experiments provide valuable insights into the germination requirements of species, it is essential to recognize that these findings may not fully capture the variability that exists within species populations. Collections of seeds or reproductive materials from different geographic locations or ecological conditions can exhibit variations in germination behavior due to genetic, environmental, or other factors (Baskin & Baskin, 2014; Daskalakou et al., 2022). *Alnus glutinosa* collected from two distinct locations, in central and northern Greece, exhibited notable differences in germination behavior, particularly in terms of the temperature range favorable for germination. These differences were also accompanied by a significant variation in seed mass, with the seeds from central Greece having approximately twice the mass of those from northern Greece. Seed mass of *A. glutinosa* sampled from many locations has been correlated with precipitation (Gomes Marques et al., 2022). In central Greece, the lowest germination temperature of untreated seeds was observed at 30°C, while in northern Greece the lowest germination temperature was at 15°C, as is also the case for seeds collected in Britain (Gosling et al., 2009). The aforementioned differences in temperature requirements for germination help to explain the timing of germination in the field, as has also been shown in other tree species (Thanos et al., 2010). If seeds from central Greece were able to germinate at low temperatures (15°C), they would sprout in autumn or winter right after seed dispersal, which would be detrimental to seedling survival. Conversely, in northern Greece and northern Europe, the temperature does not reach 15°C during seed dispersal, delaying germination until spring or early summer.

Another common characteristic among all four species is their remarkably high germination rate, with the entire germination process completed in less than 1 week, often within 4–7 days. This swift germination underscores their ability to quickly establish and adjust to their surroundings.

The light response varied among the studied species, ranging from overall light-requiring to light-promoting or light-indifferent depending on temperature. Both *Alnus* species exhibit a photorequirement, relying mainly on the presence of light for germination. Conversely, *L. orientalis* and *P. orientalis* exhibit predominantly photoindifference, although light promotion is observed under unfavorable temperature regimes. Öztürk et al. (2008) note that seeds of *L. orientalis* collected in Turkey generally exhibit indifference to light. However, to our knowledge, there has been no comprehensive study on the effect of light on the germination of the studied species.

Light requirement appears to be associated with seed mass: *Alnus* species produce seeds with much lower mass compared with *L. orientalis* and *P. orientalis*. These findings align with relevant scientific studies indicating that larger seeds are less reliant on light for germination than smaller seeds (e.g., Koutsovoulou et al., 2014; Milberg et al., 2000). All studied species share similar ecological requirements and are found in comparable riparian habitats. However, the light-requiring *A. glutinosa* and *A. incana* are also nonskotodormant or photodormant (following FR illumination), meaning they can readily germinate in gaps, canopy openings, or following soil disturbance. This conclusion aligns with their characterization as fast-growing, pioneer species, capable of colonizing disturbed areas (San-Miguel-Ayanz et al., 2016). For small-seeded pioneer species, light constitutes a gap detection signal compared with fluctuating temperatures for larger-seeded species (Pearson et al., 2002; Xia et al., 2016).

The three collections of the two *Alnus* species studied showed a rather high proportion of empty seeds, exceeding 60%. It is noteworthy that a substantial proportion of empty seeds and suboptimal germination percentages (<50%) are recurrent traits across various *Alnus* spp. (Gosling et al., 2009; Harrington et al., 2008; Kajba & Gracan, 2003), diminishing the quality of seed collections (Aránzazu-Prada & Arizpe, 2008). For *L. orientalis*, the proportion of empty seeds was quite low, at 7%. An annual seed production pattern, with abundant seed crops occurring every 3 years has been proposed for *L. orientalis* based on results from *L. styraciflua* (Aránzazu-Prada & Arizpe, 2008).

Considering that seeds of the studied species are dispersed during late autumn or early winter, the combination of high temperatures and cold stratification required for germination minimizes autumn germination and ensures that abundant germination occurs as soon as temperatures rise in spring. Moreover, this suggestion is reinforced by the absence of thermodormancy in the seeds studied. This adaptive germination strategy enables the seeds to synchronize their germination with favorable environmental conditions, promoting successful establishment and growth. The same strategy has been previously suggested by Gosling et al. (2009) for *A. glutinosa*.

Seed viability was maintained at optimal level for all four species, both after desiccation and after desiccation and storage at −20°C for 3 months, and was characterized by orthodox storage behavior. Orthodox storage behavior has been documented for *L. orientalis* and *P. orientalis*, whereas for the two *Alnus* species, it has been reported with a potential for such behavior (SER, INSR, RBGK, Seed Information Database [SID], 2023). These results demonstrate that seeds of all four species can be collected and reliably stored in seed banks, ensuring their availability for conservation and future forest restoration initiatives.

Overall, understanding the specific germination requirements and behaviors of species is essential for conservation efforts and successful restoration actions. These actions aim to preserve their genetic diversity and ability to adapt to changing climatic conditions.

5 | CONCLUSION

The germination behavior of the four riparian trees studied, *A. glutinosa*, *A. incana*, *P. orientalis*, and *L. orientalis*, in terms of temperature and light requirements, reveals a mechanism that ensures germination is delayed until spring. Seeds are dispersed in late autumn or early winter. The combination of high temperatures required for germination in autumn, cold stratification to expand the temperature range for germination, and, in some cases, light requirements, prevents autumn germination and ensures germination once temperatures rise in spring. These findings support the development of effective conservation management plans and restoration strategies for sensitive riparian habitats.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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