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Limiting temperatures of embryo growth and seed germination of *Fraxinus excelsior* L. (Oleaceae): a threshold model approach

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Abstract

Key message We have examined thermal control for embryo growth and seed germination in European ash. Using historical data (span of 36 years) of embryo index in matured seeds, we showed that maturation is not influenced by the annual average temperature. Secondary dormancy in ash's seeds is easily induced, and the thermal window for both embryo growth and seed germination was between 10 and 20 °C.

Context Seed germination plays a crucial role in establishing plant communities. Ash seeds are characterized by deep morphophysiological dormancy, usually overcome by 32 weeks of stratification. Observed climate changes might disrupt ash's thermal window for embryo growth and seed germination. Thus, proper examination of seed adaptability can be important for forest management.

Aims We investigated the effect of temperature on the embryo index (ratio between embryo and seed length) as well as embryo growth and germination of European ash (*Fraxinus excelsior* L.) seeds under different thermal conditions.

Methods We used seeds collected at the same site in Poland between 1985 and 2021 to first analyze the influence of the growing season on the initial embryo index in mature seeds. Subsequently, we examined embryo growth and germination of seeds at constant temperatures of 3°, 10°, 15°, 20°, 25°, and 30 °C from seeds collected in 2021. Using the modeled rate of embryo growth and germination, we predicted the cardinal temperatures for those processes in ash seeds.

Results The embryo index of ash seeds varied; however, there was no relationship between the index and year of seed collection. The base temperature for embryo growth was 1.4 ± 1.1 °C, with a ceiling temperature of 29.9 ± 2.0 °C and an optimal temperature of 15.7 ± 0.3 °C. The base temperature for germination was at -1.8 ± 0.3 °C. Due to the induction of secondary dormancy, the ceiling temperature of germination was impossible to establish.

Conclusions The initial embryo index of mature ash seeds seems to be an individual trait and was not affected by local average annual temperatures. The most effective temperature ranges for embryo growth and germination were similar, however narrow (between 10 and 15 °C). Temperatures above 20 °C induced secondary dormancy,

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hampering embryo growth as well as germination. Such a narrow thermal window for embryo growth can potentially act as a limiting factor for the natural regeneration of ash seeds.

Keywords Germination niche, Seeds, Threshold models, Cardinal temperatures, Embryo growth

1 Introduction

Seed germination plays a vital role in plant regeneration in the ecosystem, controlling the assembly of plant communities. Once the germination process starts, it cannot be reversed, and the newly emerging seedling is the most vulnerable stage in the plant life cycle (Donohue et al. 2010). Thus, proper germination timing ensures plant survival. The bet-hedging strategy for germination is dormancy, which allows plants to distribute germination across time (Willis et al. 2014). Dormancy and later germination are controlled by environmental cues such as light, nitrogen, and, most importantly, water availability and temperature (Bewley et al. 2013). The temperature window for dormancy release and germination for a given species helps us to describe its potential germination window (Alvarado and Bradford 2002; Daibes et al. 2019; Carta et al. 2022).

Analyzing the temperature window for germination is possible using thermal models, which describe germination rates at different temperatures. Suboptimal germination temperatures are described by increasing the germination rate and range between the minimal germination temperature, the so-called base temperature (T_b), and the optimal temperature (characterized by the highest germination rate; T_{opt}). Similarly, a supraoptimal temperature range of germination lies between the optimal and maximal germination temperatures, namely, the ceiling temperature (T_c) (Garcia-Huidobro et al. 1982; Alvarado and Bradford 2002). This approach is versatile and used to model the effect of various environmental factors (i.e., temperature, light, water) on the developmental rates of different processes (i.e., dormancy release, germination, emergence, bud opening, or flowering; Pritchard et al. 1996; De Melo-Abreu et al. 2004; Jinks et al. 2006; Daibes et al. 2019). We may then define thermal time (θ) as the accumulation of the “thermal signal” required for the seed population to attain a specific percentage of germination, typically 50% (Alvarado and Bradford 2002).

Thermal limits for dormancy and germination are valuable traits for modeling seed germination in the field as well as for studying population differences, adaptation, and the effect of climate change on plants and seeds (Fernández-Pascual et al. 2019). Dormancy release under an altered climate can either facilitate later germination (Jastrzębowski et al. 2021) or slow it down, depending on the species and population (Orrù et al. 2012). Fernández-Pascual et al. (2019) highlight that thermal time traits

depend on thermal history (temperatures experienced by plants previously in their environment) and can influence the following generation's traits (such as seed size, mass, or number) as an adaptation.

European ash (*Fraxinus excelsior* L.) is a common but scattered hardwood species known as a source of high-quality timber for making furniture. European ash is widely distributed in Europe, and its range limits are set by temperature requirements in the east and north and water availability in the south; it is found from the south of Scandinavia to northeastern Spain, the south of Greece, and Ladoga Lake in the east. It is also present on the Black Sea coast (Dobrowolska et al. 2011). Seeds are ca. 4-cm samaras, attached in bunches, usually dispersed by wind after the winter. Seeds are produced annually, with heavier crops every 2 to 5 years (Suszka et al. 1996; Dobrowolska et al. 2011). Seeds display deep simple morphophysiological dormancy (MDP), in which an undeveloped (small differentiated) embryo has physiological dormancy (Tylkowski 1991; Suszka et al. 1996). Under natural conditions, seeds germinate in the second spring after dispersal. The ratio between embryo and seed length is called the embryo index (Fig. 1), and in mature ash seeds, it is usually between 0.4 and 0.6. However, the initial embryo index can differ depending on the origin of the seeds, as seeds from the southern area of the natural range have a higher embryo index between 0.7–0.9

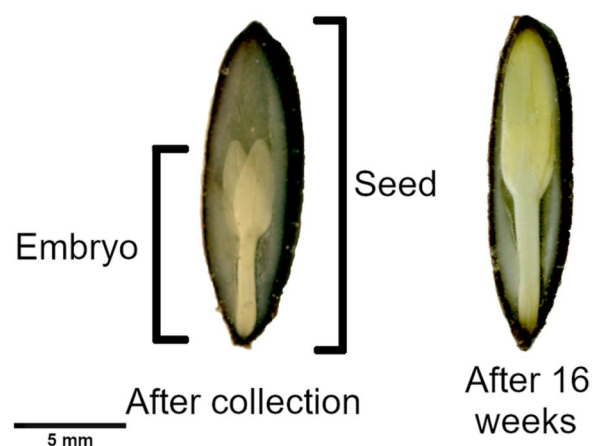


Fig. 1 Cross section of European ash seed, representing the ratio between embryo and seed length (embryo index). **A** The initial embryo index after collection. **B** Embryo index after 16 weeks of warm stratification (at 15 °C). Bar = 5 mm

(Tylkowski 1988). Recently, the ash population in Europe was depleted by ash dieback (caused by *Hymenoscyphus fraxineus* Baral) and the emerald ash borer (*Agrilus planipennis*, Fairmaire) beetle, which are the two most dangerous pathogens and pests for ash trees and which are spreading rapidly across the distribution area of ash (Dobrowolska et al. 2011).

We aimed to investigate the effect of temperature on embryo growth and germination of ash seeds. First, we examined the effect of local changes in annual means of temperature on the initial embryo index in mature ash seeds between 1985 and 2021. We hypothesized that the average annual temperature during maturation would affect the initial embryo index of seeds. Secondly, we examined the thermal requirements for embryo growth in dispersed ash seeds, followed by describing the thermal parameters of seed germination (cardinal temperatures). We hypothesized that the temperature window for embryo growth is wider than for germination.

2 Material and methods

2.1 Plant material

To examine the initial embryo index (i.e., the ratio between embryo and seed length; Fig. 1) in seeds of European ash, we used desiccated samaras stored at -3°C that were collected between 1985 and 2021 from 1–4 trees growing alongside a road in the outskirts of Poznań, Poland (Appendix Table 3; $N=21$). The initial embryo index was measured in the lab. All available initial parameters of seeds, namely, viability (via TTC test) and embryo index, can be found in Appendix Table 3. For embryo growth analysis and germination trials, we used seeds collected during 2021 (Table 1).

2.2 Embryo growth analysis

To analyze embryo growth, samaras were kept in closed boxes with an air moisture of approximately 90% and a moisture content (MC) of seeds of approximately 55% (as described in Tylkowski 1991) at constant temperatures of 3° , 10° , 15° , 20° , 25° , and 30°C . The embryo index was recorded from 40 seeds (4 replicates per 10 seeds from each tested tree; incubation boxes served as replicates). Each week, seeds were cut along the longitude line of the seed, and using a binocular microscope with a ruler, the

embryo index was measured. After 16 weeks, recorded increments were used to model embryo growth with the *grofit* package (Kahm et al. 2010; Appendix Table 4). Subsequently, the slope parameters of the obtained curves representing the growth rate were plotted against the temperatures used, to obtain the temperature range for embryo growth. Model parameters can be found in Appendix Table 5.

2.3 Stratification and germination trials

Seeds were stratified, mediumless in closed plastic boxes, first at a warm temperature (15°C) for 16 weeks and then at a cold temperature (3°C) for an additional 16 weeks (Tylkowski 1991). A high humidity was maintained within the boxes (ca. 90%), and any seeds infected by fungi were discarded. After stratification, seeds were germinated at constant temperatures of 3° , 10° , 15° , 20° , 25° , and 30°C . Seeds were sown on moisture filter paper and placed on a Petri dish, with 25 seeds in 4 replicates from each treatment and tree. Seeds were considered germinated when the radicle exceeded 3-mm length. Seeds were counted daily for 31 days. At the end of germination, non-germinated seeds were cut to eliminate empty or decayed seeds.

The total germination percentage (G%) was computed as the proportion of germinated seeds in relation to the total number of viable seeds sown in Petri dishes. The cumulative G% of seeds was plotted against time and fitted to a four-parameter Hill function (El-Kassaby et al. 2008) to obtain time to reach 50% germination as well as germination time for successive increments of 10% germination (10 to 90%). The germination rate ($1/t_x$) of those nine percentiles was regressed against the germination temperature, separately for below and, if possible, above the optimum temperature. The obtained regressions were used to establish T_b , T_{opt} , and T_c . The inverse of the slope of the suboptimal regression lines of each percentile was calculated separately to estimate the thermal time (θ , $^{\circ}\text{Cd}$). Subsequently, percentage data were transformed to probit and regressed against thermal time, which allowed calculating the thermal time required for 50% of viable, nondormant seeds to germinate (see Sampayo-Maldonado et al. 2019).

Table 1 Initial information about collected European ash seeds used for estimation of embryo growth and germination

Localization	Name	Collection date	Embryo index
Tarnowska Poznań, Poland $52^{\circ}20'28.9''\text{N}$ $17^{\circ}00'29.2''\text{E}$	Tree no. 1	8 December 2021	0.47 ± 0.05 (c)
Tarnowska Poznań, Poland $52^{\circ}20'28.9''\text{N}$ $17^{\circ}00'29.2''\text{E}$	Tree no. 2	8 December 2021	0.61 ± 0.07 (a)
Zwierzyniec Kórnik, Poland $52^{\circ}14'58.0''\text{N}$ $17^{\circ}04'32.2''\text{E}$	Tree no. 3	9 December 2021	0.54 ± 0.05 (b)

Mean $\pm$ SD; one-way ANOVA, followed by post hoc Tukey's test, was carried out; means with the same letter are not different at $p < 0.05$

2.4 Statistical analyses

All analyses were performed using R software (R Core Team 2022). For the estimation of the nonlinear curve for embryo growth, the *grofit* package was used (Kahm et al. 2010). The germination curve was modeled using the *germination metrics* package (Aravind et al. 2023). The correlation between climate parameters and embryo index was calculated using Pearson's method. Differences between germination at different temperatures were assessed using ANOVA and post hoc Tukey's tests. The normal distribution of residuals was assessed using the Shapiro–Wilk test, and homogeneity of variance was assessed using Levene's test. The datasets generated and the code used for the analyses during the current study are available in Figshare repository (see Wawrzyniak et al. 2023).

3 Results

The mean embryo index of seeds collected between 1985 and 2021 was 0.57 (Fig. 2). During this period, no relationship between year and embryo index was found ($R^2 = 0.01$; $p = 0.78$).

Growth at all examined temperatures was fitted to logistics models except for the temperature of 3 °C, for which only a linear model could be fitted (Appendix Table 3). Embryo growth was the most dynamic between 10 and 20 °C, and the embryo index increased between 0.22 and 0.3 after 16 weeks in all tested trees (Fig. 3). The lowest embryo index increment was ca. 0.04 at 3 °C (Fig. 3). The highest embryo index increment was at 15 °C (0.3), and no significant differences in the embryo index increment were observed between 15°, 10° (0.29),

and 20 °C (0.24; Fig. 3). Despite differences in the initial embryo index in the collected seed lots (Table 1), the increment in the embryo index was similar between the tested trees (Table 2).

The relationship was characterized by a polynomial regression model ($R^2 = 0.74$, $p \leq 0.000$). The highest embryo growth rate was reached at a temperature of 15.7 ± 0.3 °C. The base temperature for embryo growth was 1.4 ± 1.1 °C, and the ceiling temperature was 29.9 ± 2.0 °C. The growth rate differed between the tested trees at temperatures above 10 °C (Fig. 4).

Final germination values differed between examined trees as well as the temperature used during incubation. At 10 °C, seeds of tree number 1 germinated to 63%, tree number 2 to 95%, and tree number 3 to 57% (Fig. 5A). Generally, seeds from all tested trees germinated significantly better at lower temperatures, between 3 and 15 °C. Seeds at higher temperatures (mainly 25° and 30 °C) remained viable, but germination either stopped at a low percentage (ca. 40% in tree number two and ca. 5% in tree number one) or did not occur (tree number three). At 20 °C, seeds germinated slightly better than at higher temperatures (64% in tree number 2 and 11% in tree number 1). Germination at 3 °C was the slowest in all tested trees (between 11 and 22 days; Fig. 5B). Seeds in which dormancy was mostly overcome at first germinated at a similar speed but eventually stopped due to induction of secondary dormancy at higher temperatures (above 20 °C). Only seeds at temperatures below 20 °C germinated to their full possible capacity.

In order to calculate cardinal temperatures, the rate of germination was analyzed against temperatures.

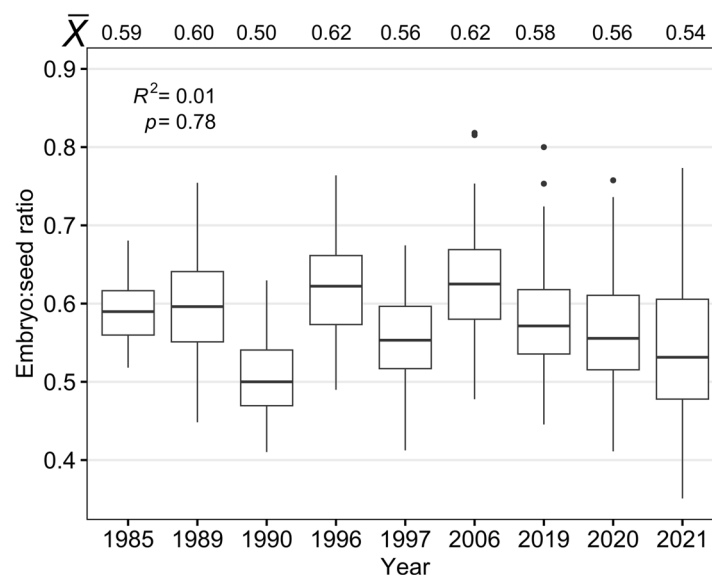


Fig. 2 Embryo index of European ash seeds after collection from 1985 to 2021. Upper values represent the mean EI value ($\bar{x}$)

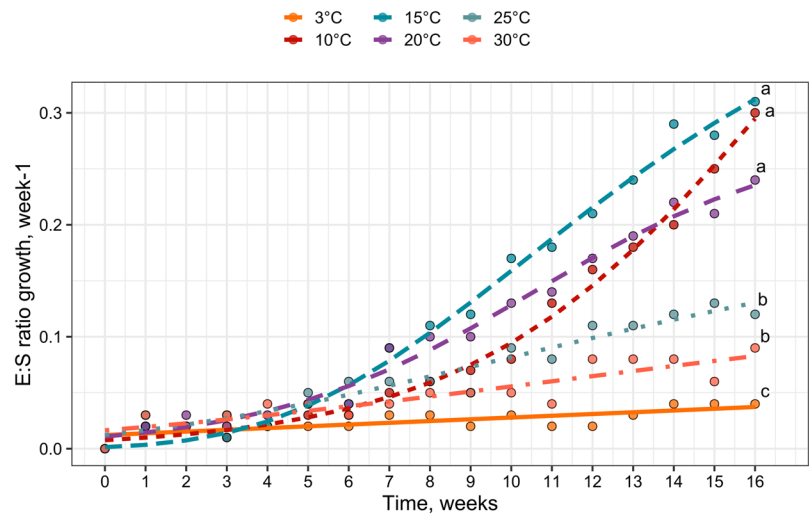


Fig. 3 Weekly increment of embryo index at constant temperatures: 3°, 10°, 15°, 20°, 25°, and 30 °C. Lines represent logistic or linear models fitted to embryo growth. The parameters of the modeled curves are shown in [Appendix Table 4](#). For each result after 16 weeks of warm stratification, one-way ANOVA, followed by post hoc Tukey’s test, was carried out; means with the same letter are not different at $p < 0.05$. Means $\pm$ SEs. $N = 3$ replicates

Table 2 Analysis of variance results from increasement of embryo index after warm stratification at $p < 0.05$

Factor	Df	F-value	<i>p</i>
Temperature	5	76.30	<0.000
Tree	2	2.16	0.126
Temperature $\times$ tree	10	1.3	0.252
Residuals	54		

Significant differences are bolded

However, treatments for which germination capacity was below 30% were excluded from the analysis (Fig. 5B). Therefore, a reliable estimation of optimal and ceiling temperature was impossible due to low germination at higher temperatures (20–30 °C). The base temperature was estimated to be -2 ± 0.5 °C in tree number one and -1.6 ± 0.3 °C in tree number 2.

In tree number 1, the thermal time θ for 50% germination was 132.3°Cd, and for tree number 2, it was 53.8°Cd (Fig. 6). Such a difference was a result of a stronger dormancy level, which remained in tree number one seeds.

4 Discussion

Due to ongoing climate change, a steady increase in air temperature has been observed in recent decades on both regional and continental scales. However, these changes are not evenly distributed; warming is expected to be larger at higher latitudes, with more severe consequences (Venäläinen et al. 2020). In Poland, an increase

of a number of days with active temperatures due to the extended growing season can be observed (Wypych et al. 2017). Examining long-term data from local weather stations shows a similar trend at our study site ([Appendix Fig. 7](#)). These changes affect plant phenology, which is often considered an early bioindicator of climate change and is usually associated with an earlier start of the growing season (Piao et al. 2019; Menzel et al. 2020). In general, changes in reproductive phenology will further affect subsequent life stages, disrupting seed dormancy, dispersal, and germination timing. However, the magnitude and direction of those changes will differ depending on species and habitat (Fernández-Pascual et al. 2019). The embryo index of mature ash seeds at collection differs depending on the latitude. Tylkowski (1988) describes that seeds collected across the natural distribution of ash in the north had a lower embryo index at the time of maturation (0.4–0.5) than seeds collected from the south (0.7–0.9). Seeds with a higher embryo index germinate earlier as the time required for embryo growth was shorter. The effect of climate conditions explained those differences. Despite increasing temperature in Poland, the embryo index of ash seeds collected in Poland between 1985 and 2021 showed no correlation with the year of collection. The embryo index of the collected seeds was between 0.5 and 0.6 in all examined years. According to Tylkowski (1988), the growth of ash embryos stops in the milky-waxy stage ca. 4 months after flowering. For *orthodox* seeds (i.e., desiccation tolerant),

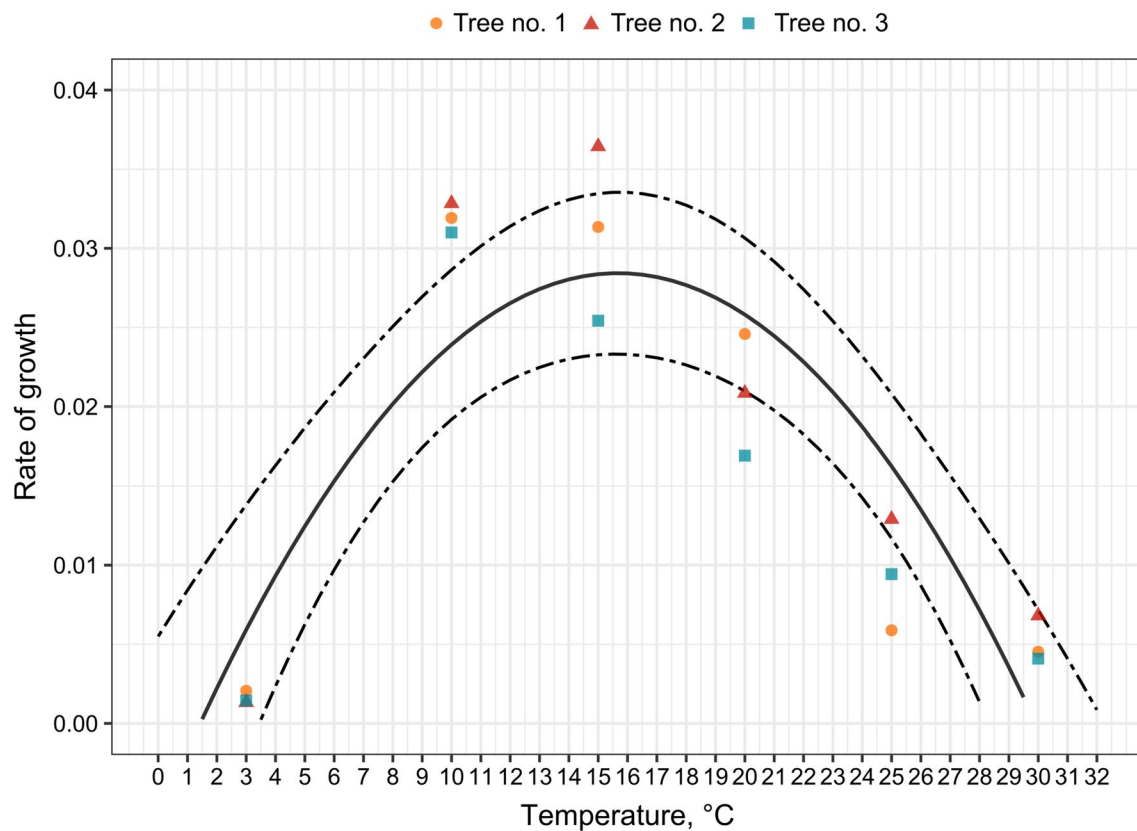


Fig. 4 Growth rate of embryos. The dotted line represents 95% confidence intervals. Model parameters are in [Appendix Table 5](#). Intercepts of the x-axis represent the minimum ($T_b = 1.4 \pm 1.1$ °C) and maximum temperature ($T_c = 29.9 \pm 2.0$ °C) for embryo growth, and peak represents optimal temperature ($T_{opt} = 15.7 \pm 0.3$). Points represent tested trees

drying and, therefore, metabolism decrease are the normal process when maturation approaches completion (Bewley et al. 2013). Moreover, small and scattered populations of European ash in Europe are characterized by high genetic differentiation between them; however, this differentiation is low within the same populations of European ash (Heuertz et al. 2004b, 2004a). Therefore, the embryo index might not be influenced by climate during maturation but may be a trait of a given genotype. However, to support this claim, more studies on the embryo index of seeds of different ash populations must be conducted. Due to the limits of historic data availability of embryo index of ash seeds, our study was restricted only to a small population of trees. The analyzed group of trees was relatively small; however, it was uniform which allowed to minimize the interpopulation differences. In 36-year span of analyzed data, the initial embryo index remained around 0.5, which is coherent with the information available in the literature.

Seeds of European ash usually require 16 weeks of warm-phase (15–20 °C) stratification followed by cold-phase (1–7 °C) stratification for an additional 16 weeks. Warm-phase allows embryo growth to be finished up to 0.8–0.9 of the embryo index, and the cold phase alleviates intrinsic physiological dormancy. Depending on the origin of the seeds, those time frames might differ, as embryo index at maturation and dormancy depth varies. In favorable conditions, seeds of European ash germinate in the second spring after dispersal. The growth of embryos after dispersal is controlled by water availability and temperature. Morphophysiological dormancy has been described in other temperate forest species, such as *Aconitum lycoctonum* L. (Vandelook et al. 2009), *Cardiocrinum giganteum* (Wall.) Makino (Phartyal et al. 2012), *Corydalis ambigua* Cham. and Schltld. (Kondo et al. 2005), *Aquilegia barbaricina* (Arrigoni & E. Nard.) (Porceddu et al. 2017), *Ilex* spp. (Galíndez et al. 2018), and *Taxus*

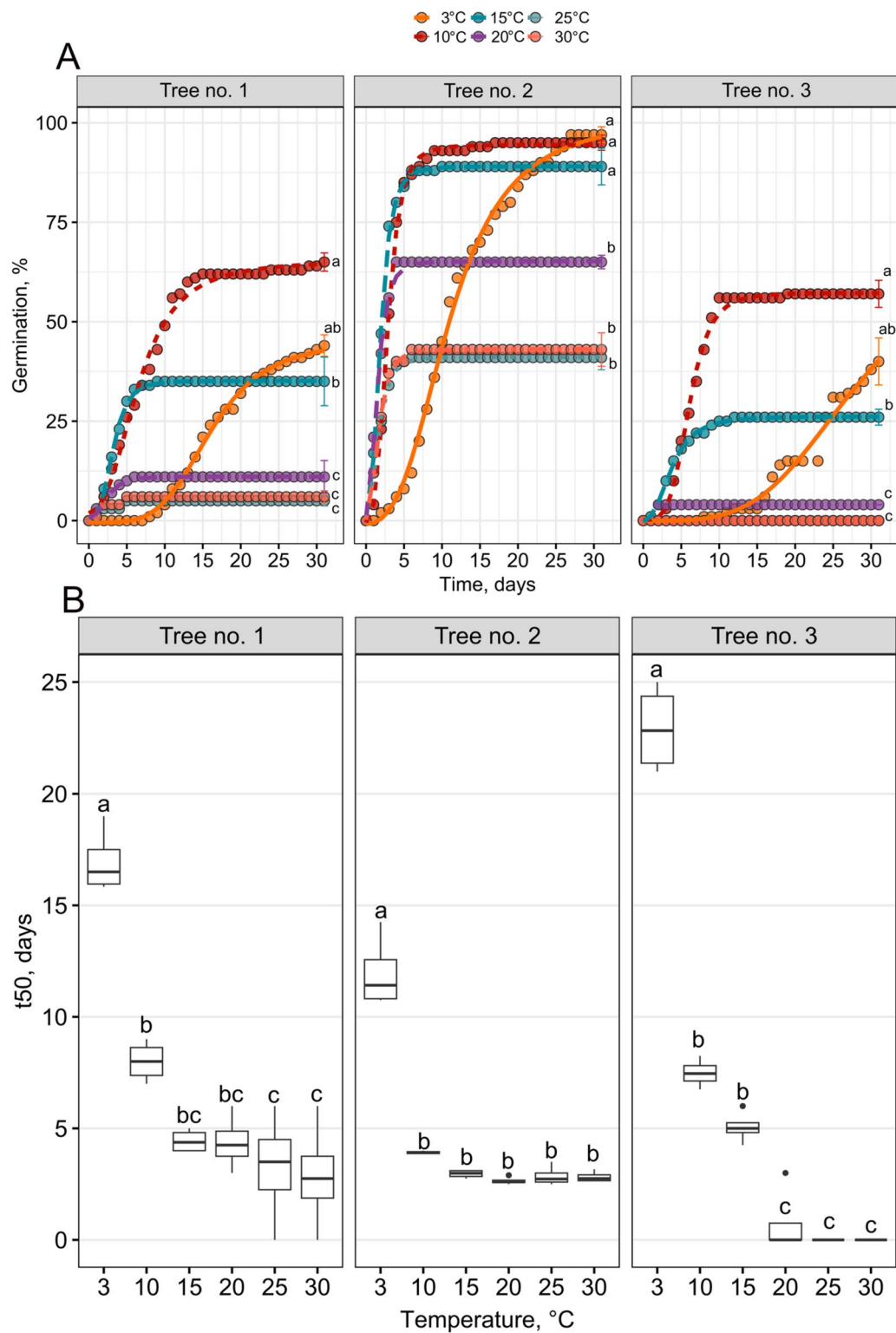


Fig. 5 **A** Germination of seeds collected from three trees of European ash at six constant temperatures (3°, 10°, 15°, 20°, 25° and 30 °C). The germination curves were fitted using a four-parameter Hill function. **B** Median time to reach 50% of final germination. For each result, one-way ANOVA, followed by post hoc Tukey's test, was carried out; means with the same letter are not different at $p < 0.05$. Means $\pm$ SEs. $N=4$ replicates

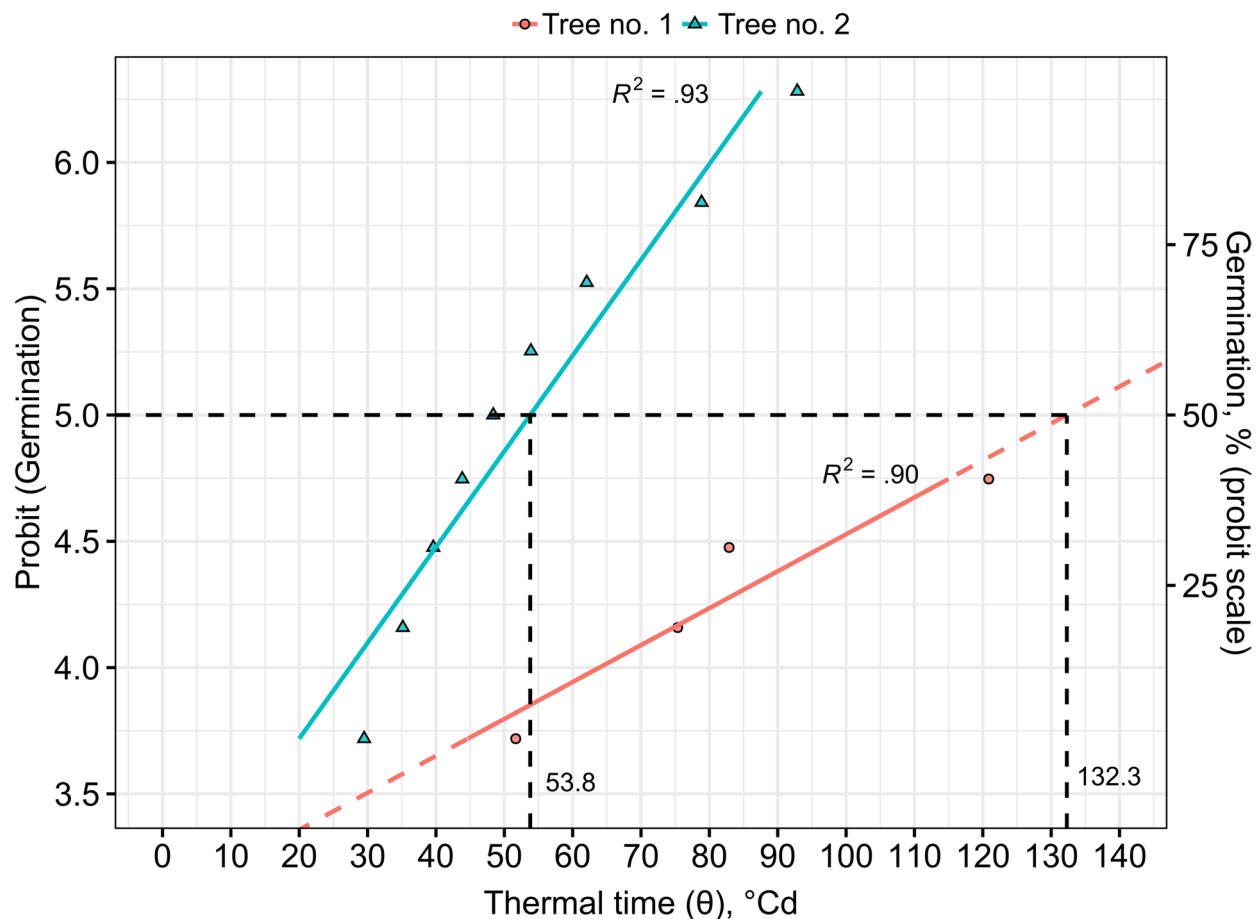


Fig. 6 Thermal time for germination of seed collected from two European ash trees, with T_b values at -2.0 °C and -1.6 °C, respectively. Dashed line indicates thermal time required for the germination of 50% of seeds

baccata L. (Pers-Kamczyc and Suszka 2022). Although the collected European ash seeds had different embryo index values after their maturation, the embryo index increase was similar after 16 weeks of incubation at a given temperature (up to 0.3). However, depending on genotype, the rate of embryo growth differed at temperatures above 10 °C. Additionally, it was reported that seeds of *A. nemorosa* showed differences in embryo growth rates. However, those differences were correlated with growth habitat rather than initial embryo index value (Mondoni et al. 2008). For European ash, we estimated the base temperature for growth at 1.4 °C. The highest rate of embryo growth was observed at 15 °C, and the ceiling temperature was estimated at 29.9 °C. Similar cardinal temperatures of embryo growth were reported for *Apaiddia barbarica* at T_b 5.2 °C, T_{opt} 15 °C, and T_c 29.5 °C (Porceddu et al. 2017). On the other hand, *A. lycoctonum* embryo growth was observed at temperatures below 10 °C

(Vandelook et al. 2009). *Campanile giganteum* embryo growth was observed at alternating temperatures, ca. 20 °C and followed by colder temperatures ca. 10 °C (Phartyal et al. 2012). Embryo growth in *T. baccata* seeds was observed almost exclusively in alternating temperatures (24/24-h cycles) only between a narrow range of temperatures, between 15 and 20 °C (Suszka 1985). Embryos of *Thalictrum rochebrunianum* H. Lev. endemic to the Korean peninsula require lower temperatures for embryo growth, ca. 5 °C (Lee et al. 2015). Although not tested in this experiment, additional slow growth of the embryo is possible during cold-phase stratification, which is consistent with what was reported previously (Tylkowski 1991).

Germination timing depends on the same environmental cues as dormancy release (mainly temperature and water availability). Estimating the threshold values for germination can set the thermal limits of species in a given environment and allow us to estimate the

start of germination. Due to insufficient germination of seeds at higher temperatures, we could estimate T_b only in tree number 1 and number 2 and T_{opt} for tree number 2. T_b was estimated at -2° and -1.6°C in tree numbers 1 and 2, respectively. Small differences between T_b of tested trees could be result of individual traits of seeds (i.e., size, dormancy depth) which influence the germination rate (Xu et al. 2016; Moosavi et al. 2022). This result corresponds with T_b found for European ash by Jinks et al. (2006) at 0.12°C , from trees collected in the UK. In general, seeds of trees native to Northern Europe had similar, low T_b , i.e., *Alnus incana* L. (1°C), *Betula pubescens* L. (1°C), *Pinus sylvestris* L. (0°C) (Milbau et al. 2009), *Quercus robur* L. (1.5°C), *Castanea sativa* L. (1.4°C) (Pritchard and Manger 1990), *Acer pseudoplatanum* L. (Jinks et al. 2006), and *Acer platanoides* L. (-2.6°C) (Jensen 2001). Low T_b allows them to germinate relatively early in spring; however, seedlings might exhibit frost damage. Dry seeds are usually characterized by the highest frost resistance, dramatically decreasing in seedlings and juvenile plants (up to eightfold in some alpine species) (Marcante et al. 2012).

Germination in ash seeds occurs at 53°Cd in seeds without dormancy, and it might be higher for seeds where dormancy was not fully released. However, T_c was impossible to establish due to little or no germination at $\geq 20^\circ\text{C}$. For the same reason, reliable T_{opt} was difficult to obtain; however, obtained germination rates show that optimum temperature for ash seed germination settled around 15°C . Temperatures $\geq 20^\circ\text{C}$ induced secondary dormancy, which inhibited further germination. Induction of secondary growth can significantly decrease seed germination both in the laboratory and in the field. Using constant temperatures of 20°C for *Carpinus betulus* L. seeds decreased germination to almost 20% compared to treatment with alternating temperatures (Suszka et al. 2021). Similar problems were reported by Jinks et al. (2006), where a rapid increase in soil temperature stopped seed emergence of European ash and *Acer pseudoplatanus*, forcing seeds to remain in the soil for another year. It has been shown that climate change increases soil temperature with increasing air temperature and reduction in snow cover (Qian et al. 2011; Potopová et al. 2021). However, it is not certain at this stage how climate change will affect the germination of European ash seeds. It is believed that European ash distribution under different climatic scenarios is not threatened; instead, the distribution is projected to expand (Dyderski et al. 2018). Temperature affects dormancy break and germination, making

both effects difficult to distinguish in laboratory tests. Despite that, nondormant seeds of European ash germinated rapidly at almost all examined temperatures (10° , 15° , 20° , 25° , 30°C), and total germination capacity was reached only at temperatures $\leq 15^\circ\text{C}$. A temperature of 15°C sets a dormancy loss threshold, above which secondary dormancy is induced. Such thresholds for dormancy induction can be modeled; however, a wide range of dormancy release time in seeds population makes it difficult to set a reliable and easy-to-observe threshold that could be used for modeling (Pritchard et al. 1996; Batlla and Benech-Arnold 2015; Masin et al. 2017).

5 Conclusions

The results do not support our hypothesis, as the initial embryo index of mature European ash seeds was unaffected by local climate anomalies. In 36-year span of analyzed data, the initial embryo index remained about 0.5, proving to be a relatively conservative seed trait. Differences described in literature, in the embryo index between northern and southern ash populations, might be a result a stronger and longer natural selection. However, more analyses, including more population across the wide distribution range of European ash, are needed to assess the genetic influence on the initial embryo index. The temperature ranges controlling embryo growth (during dormancy release) and germination were similar. Temperatures $\geq 20^\circ\text{C}$ resulted in significantly lower embryo growth and induced secondary dormancy, which limits the thermal window to narrow range of $10\text{--}15^\circ\text{C}$. Temperature 15°C overlapped both dormancy release and fast germination, which seems most suitable for germination. Seeds of European ash were shown to be well adapted to colder regions with climates characterized by strong seasonality, as nondormant seeds germinate quickly and synchronously in temperatures $\geq 10^\circ\text{C}$, needing 53°Cd to germinate 50% of viable seeds. Therefore, the main obstacle to natural regeneration of European ash from seeds would be dormancy break conditions, rather than germination conditions. A relatively long period of dormancy release postpones the germination of ash seeds to the second spring after maturation. Changing environmental cues (i.e., disappearing snow cover and increase of soil temperature in spring) might favor ash seeds with less deep dormancy (higher initial embryo index), as seeds with deeper dormancy may not germinate before higher temperature hampers their germination, leaving seeds for another season in soil.

Appendix

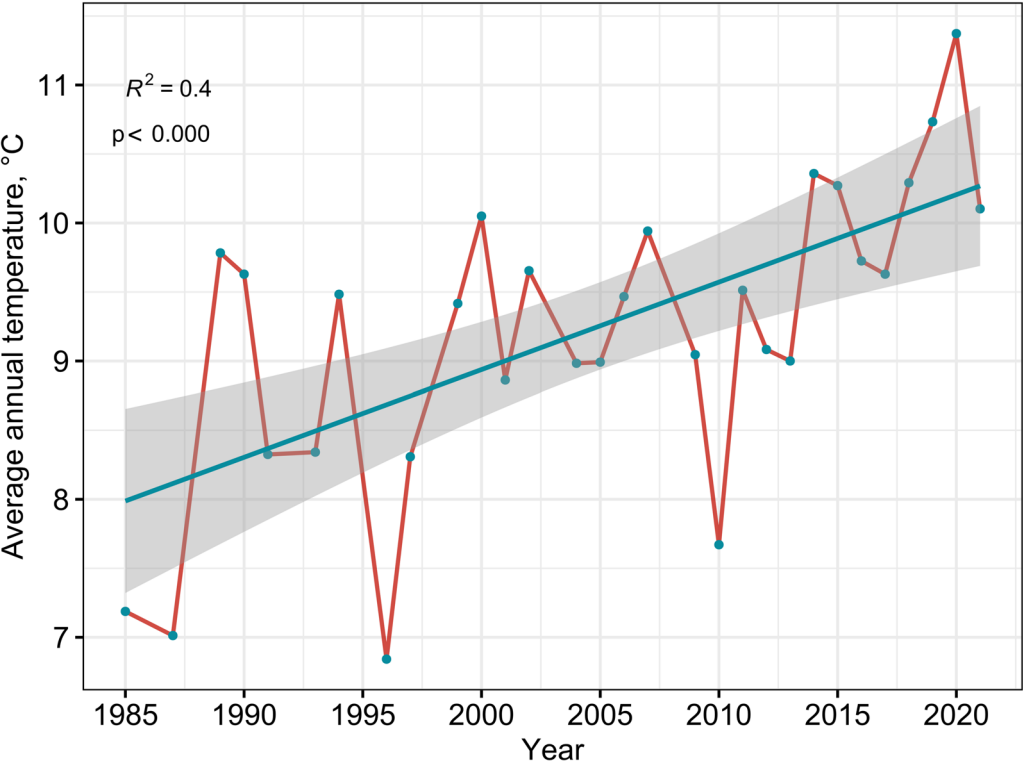


Fig. 7 Time course of the average annual temperature between 1985 and 2021. Data retrieved from Institute of Meteorology and Water Management-National Research Institute, Poland. Data recorded by meteorological station at Kórnik

Table 3 Localization and basic characteristics of European ash seeds, collected between 1985 and 2021

Localization of trees	Collection date	Viability (TTC), %	Embryo index	N (seeds)
Tarnowska, Poznań, Poland 52°20'28.9"N 17°00'29.2"E	November 1985	90.5	0.59	40
	24 October 1989	97.5	0.61	40
	24 October 1989	94	0.55	40
	24 October 1989	92.5	0.62	40
	22 October 1990	98	0.5	80
	12 October 1990	-	0.52	40
	8 November 1996	92.5	0.62	120
	25 September 1997	100	0.53	40
	25 September 1997	100	0.57	40
	25 September 1997	100	0.57	40
	14 October 2006	72.7	0.62	40
	14 November 2006	-	0.63	80
	27 January 2019	-	0.55	40
	27 January 2019	-	0.61	40
	27 January 2019	-	0.56	40
	8 January 2020	-	0.59	40
	8 January 2020	-	0.56	40
	8 January 2020	-	0.58	40
	8 January 2020	-	0.52	40
	8 December 2021	-	0.47	40
	8 December 2021	-	0.61	40

Table 4 Model parameters used to examine embryo growth of European ash seeds in constant temperatures (3°, 10°, 15°, 20°, 25°, 30 °C). Model fitting was carried out using *grofit* package (Kham et al., 2010) and based on the Akaike information criterion

Temperature, °C	Used model	Model parameters				
		μ	λ	A	a	b
3	Linear	-	-	-	0.002 ± 0.001	0.01 ± 0
10	Logistic	0.04 ± 0.01	9.07 ± 1.17	0.64 ± 0.17	-	-
15	Gompertz	0.03 ± 0.00	4.44 ± 0.29	0.46 ± 0.06	-	-
20	Logistic	0.02 ± 0.00	3.99 ± 0.38	0.28 ± 0.02	-	-
25	Gompertz	0.01 ± 0.000	0.54 ± 1.01	0.22 ± 0.09	-	-
30	Gompertz	0.004 ± 0.005	-2.1 ± 3.35	0.17 ± 0.18	-	-

Table 5 Model summary of polynomial linear regression for embryo growth rate in relation to temperature

Coefficients	Estimate	p
Intercept	0.01665 ± 0.002	< 0.000
Temperature (x)	-0.00941 ± 0.007	0.188
Temperature ² (x ²)	-0.04409 ± 0.007	< 0.000
Residual error	0.0068	
Df	15	
R ²	0.7448	

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Code availability

The code used for analyses is available in the Figshare repository (<https://doi.org/10.6084/m9.figshare.23791512.v4>).

Authors' contributions

Conceptualization, MKW; conducting the experiment, MKW, JMLL, and JPRM; writing—original draft preparation, MKW; writing—review and editing, MKW, JMLL, JPRM, and PC; data analysis, MKW; and supervision, MKW. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

The datasets generated and analyzed during the current study are available in the Figshare repository (<https://doi.org/10.6084/m9.figshare.23791512.v4>).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors gave their informed consent to this publication and its content.

Competing interests

The authors declare that they have no competing interests.

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