

## Seed germination traits of *Pinus heldreichii* in two Greek populations and implications for conservation

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Seed germination traits were assessed on *Pinus heldreichii* H. Christ, a Tertiary relict, high-elevation Mediterranean pine, endemic in the western Balkan Peninsula and southern Italy; it is naturally grown at the northern Greece mountains, but also found in shrubby form above the timberline. Closed and mature cones were collected (October) for three consecutive years from Pindos Range and Mt. Olympus populations. Cone and seed morphological traits were recorded along with the seedling cotyledon number. Seed germination tests were performed under various ecologically meaningful temperatures and light regimes; climate change effects on seed germination and seedling development were assessed based on two climate scenarios. Final seed germination was moderate (~55%) on both, untreated seed lots at most favourable conditions (15 and 20 °C). Although cold stratification is not an absolute requirement, seed germination rate and final percentage are promoted by both a month of cold stratification (at least 30 or 45 days) and white light. The predicted climate change suggests that a prolonged drought period (>3 months) might turn out by the end of the century in the populations studied. Although the temperature increase might not have significant effects in the germination window of the species. The rainfall decrease will inevitably expose the young seedlings to the summer drought, thus increasing the potential mortality rate. Both *ex situ* (e.g., germplasm conservation) and *in situ* measures of conservation are recommended for the species survival in Greece, with a particular focus on the populations established at lower altitudes.

**Keywords:** Climate Change, Cold Stratification, Cotyledon Number, Heldreich's Pine, Light Response, Seed Ecophysiology, Seed Mass

### Introduction

*Pinus heldreichii* H. Christ. (1863) belongs to the high-elevation pines that grows in the treeline of the Mediterranean Basin (Chritchfield & Little 1966, Krugman & Jenkinson 2008). Heldreich's pine is a Tertiary relict species (Vidakovic 1991), a long-lived tree, exceeding 1400 years of age (Klippel et al. 2017) and endemic to the mountains of the Balkan Peninsula (Christensen 1997, Vendramin et al. 2008, Farjon 2018). Its native, scattered populations are distributed from Bosnia and Herzegovina, Montenegro, Albania, Serbia, North Macedonia, and

southwest Bulgaria to northern Greece (Fig. 1), while isolated populations occur in southern Italy (Bucci et al. 1997). In Greece, *P. heldreichii* is naturally growing in the northern part of the country, at high elevations from 1300 to 2300-2600 m a.s.l., forming woodlands up to the treeline, in pure stands or mixed forests – often replacing *P. nigra* at higher altitude; it is also found in a shrubby form well above the timberline (Christensen 1997, Dimopoulos et al. 2013). It covers a relatively small area (8300 ha) growing at the southern limits of the species world distribution (Skordilis & Tha-

nos 1997) and its populations are distributed in Mount (Mt.) Olympus and the Pindos Range (Valia Kalda and Vikos-Aoos National Park, Mt. Smolikas and Zagori area), on serpentine, limestone or granite soils (Dimopoulos et al. 2013).

*P. heldreichii* is a tolerant tree to wind, ice and heavy snowstorms and therefore, in certain cases, has been used on high-altitude reforestation (Vendramin et al. 2008). Due to its very thick bark (ash-grey in young trees, yellowish-brown, splitting into rhomboid patches at maturity), twigs and shoots, in addition to the unique needle arrangement, the species is considered as a pine with a high ornamental value (Vidakovic 1991).

Although the species has a rather limited distribution, its habitats are considered important, as they constitute climax plant communities of great ecological value (Dimopoulos et al. 2013). The habitat of the species is considered as Near Threatened (NT) in the wider range of the EU28 (European Red List of Terrestrial and Freshwater habitats – Janssen et al. 2016). The species has been assessed as Least Concern (LC) at global and European level (Rivers et al. 2019), forming fragmented populations found on edaphically limited sites and presenting a continuing decline in area, extent and quality of its habitat (IUCN Red List of

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**Fig. 1** - The distribution of *Pinus heldreichii* in the Mediterranean Basin, adapted from the European Forest Genetic Resources Programme (Vendramin et al. 2008).



Threatened Species – Caković et al. 2017). Human influence on the climate system is currently well documented and the continued emission of greenhouse gases will cause further warming and long-lasting changes in all climatic components, increasing the likelihood of severe, pervasive and irreversible impacts for ecosystems (IPCC 2021). Tree- and timber-lines have received growing interest in recent decades, due to their potential role as biological indicators of climate (Deslauriers et al. 2008, Bonanomi et al. 2018, Bryn & Potthoff 2018, Vitali et al. 2019) and land use change (Gehrig-Fasel et al. 2007). Climate warming may cause shifts in the latitudinal and altitudinal distribution of species, increase extinction rates, and alter provision of ecosystem services; therefore, accurate predictions of the future potential distributions of key species, such as endemic forest trees (Cheddadi et al. 2016) are greatly needed. A temperature increase in timberline zones can have various effects, including treeline shifts (Kullman 2002, Savolainen et al. 2011), enhanced tree height growth and

higher reproductive success (Lett & Dorrepaal 2018). However, limited research has been conducted regarding climate change effects on seed germination and seedling growth but an overall negative impact of global warming on seed germination is anticipated (Thanos & Fournaraki 2010, Walck et al. 2011, Dantas et al. 2020).

Among the most important and probably devastating threats for *P. heldreichii* are (i) the potential hybridization with (planted) *Pinus nigra* J.F. Arnold (Caković et al. 2017); (ii) habitat fragmentation and reduced gene flow (Vendramin et al. 2008); (iii) intensive exploitation, road construction and tourism development; (iv) illegal cutting and grazing; and (v) wildfires and global climate change effects. A notable consequence of climatic change could be the increased wildland fire risk in high altitudes and for species not adapted to fire, e.g., *Abies cephalonica* (Daskalidou et al. 2019). Specific *ex situ* conservation actions can significantly contribute to the protection of these species.

Heldreich's pine is a monoecious tree, up

to ca 35 m tall (Vidakovic 1991, Christensen 1997), and seed production begins at the age of 15-20 years (Vendramin et al. 2008). "Flowering" occurs in spring, from May to June (or July). Female cones develop in a whorl, usually 1-2 (rarely in clusters of 3), that ripen in early autumn of the second year after pollination, from September (occasionally August) to October (Vidakovic 1991, Skordilis & Thanos 1997, Krugman & Jenkinson 2008). Seeds are grey coloured, 5-7 mm long, with a 25-30 mm long wing. Seed dispersal occurs in autumn (September-October); intervals between large cone crops (masting years) have not been documented (Krugman & Jenkinson 2008). According to the recommendations for breaking seed dormancy of the International Rules for Seed Testing (ISTA 2007), *P. heldreichii* is a species with an absolute pre-chilling requirement (42 days of cold stratification at 3-5°C).

It is well known that reproduction, seed dispersal, germination and seedling establishment constitute the most critical stages in a plant life cycle. Germination characteristics of seeds collected from different sites can vary in many ways (Baskin & Baskin 2014), and especially for *P. heldreichii*, in the degree of dormancy (Borghetti et al. 1989).

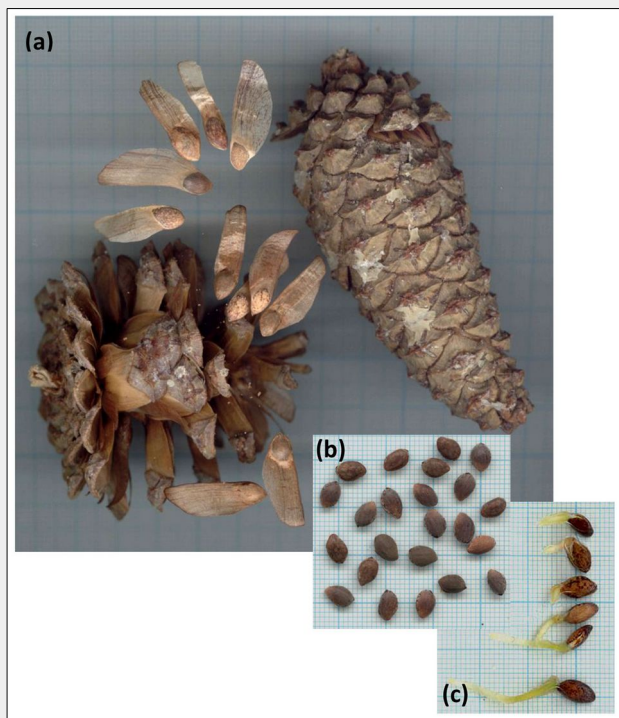
The aim of the present study is the investigation of *P. heldreichii* seed germination ecophysiology, with the use of laboratory tests performed under various ecologically meaningful temperatures, chilling treatments and light regimes. In addition, climate change effects on seed germination and seedling development are also assessed based on two climate scenarios. A three-year study of seed germinability was carried out on seeds collected from the two main *P. heldreichii* populations of Greece. Since relatively little research has been carried out on Heldreich's pine seed germination (Borghetti et al. 1986, 1989), data obtained in this study could contribute to a better understanding of germination mechanisms, seedling establishment and natural regeneration trends of *P. heldreichii* (Fady et al. 2016) concerning its scattered natural populations. Furthermore, the results of this study might be used as recommendations to both forest ecologists and managers towards performing successful germination tests and massive seedling production in nurseries, thus ensuring an effective conservation policy.

## Materials and methods

### Cone collections, biometric characteristics and seed processing

Closed, mature cones of *P. heldreichii* (n = 20-32) were gathered from ~25 randomly selected trees per collection during the maturation period (October) from two natural populations in the Pindos Range (Metsovo, Katara – 39° 48' 5.91" N, 21° 15' 11.09" E; elevation 1400 m a.s.l.) and Mt. Olympus (Katerini, Agapitos Refuge – 40° 04' 38.35"

**Fig. 2** - (a) Mature, open and closed *Pinus heldreichii* cones bearing long-winged seeds; (b) processed seeds prepared for germination tests; (c) young seedlings.



N, 22° 22' 18.11" E; elevation 2200 m a.s.l.). Cone collections were carried out by the local Forestry Departments of: (i) Metsovo in 2011, 2012 and 2013 (hereafter batches A, B and C, respectively), and (ii) Katerini in 2012 and 2013 (hereafter batches D and E, respectively). Closed cones (Fig. 2a) were left to air-dry in a sunny place at room conditions (~20 °C) until they opened (after 1-2 weeks) without any additional treatment, as recommended by Krugman & Jenkinson (2008). Fresh cone mass (g) and mass of sound seeds per cone (g) were weighed in the laboratory (Tab. 1) with an analytical balance (N34120® Navigator portable electronic balance, OHAUS, Switzerland – accuracy 0.1 mg). Seeds were extracted from the cones manually (Fig. 2b) and their wings were removed. For each cone, the total number of seeds was counted (20-130 min-max values) and the total mass of the seeds from each cone was measured; the average individual seed mass (mg) was calculated based on the total seed mass that was divided by the number of seeds per cone. Seeds of the same collection year and provenance were merged into a single batch (a total of 5 batches, A-E).

**Seed germination experiments**

Germination tests for each treatment were performed with 5 samples of 20 seeds per Petri dish (diameter 9 cm, lined with two discs of filter paper and moistened with 6 ml of deionised water). Seed germination experiments were performed in temperature-controlled plant growth chambers (model KBW 240, Binder, Tuttlingen, Germany), where temperature was kept constant within ± 0.5 °C, in either alternating light/dark conditions (L/D, photoperiod 12:12 h) or continuous darkness (D). For the experiments in continuous darkness, Petri dishes were placed in metal, light-proof containers.

Germination experiments were carried out at: (i) constant (10, 15, 20, 25 and 30 °C) and alternating temperatures (20/10 °C) for untreated (non stratified) seeds; and (ii) at constant temperatures (10, 15 and 20 °C) after various chilling durations (0, 15, 30 and 45 days) for treated (stratified) seeds. Furthermore, in the case of batch A, seeds were transferred under L/D, D and Far Red light (FR/D) at 20 °C, after 30 and 45 days of cold stratification. Daily Far Red light in the plant growth chamber was obtained by fil-

tering white light through 2 blue + 1 red plexiglass filters during the day, thus simulating the light conditions for seeds lying on the soil surface under a closed forest canopy (Thanos & Doussi 1995). Cold, moist stratification was carried out by keeping the imbibed seeds in darkness, at a temperature of 2-4 °C (in a refrigerator). A number of the treatments were not applied in certain batches (indicated with “x” in Figs. 4 and 5) as a result of limited seed availability.

As a follow-up experiment, after completing the constant temperature tests without stratification, the non-germinated seeds were further transferred, to alternating or constant temperatures under L/D (12:12 h) as follows: (i) from 10 °C (L/D and D), 15 °C (L/D) and 30 °C (D) to 20/10 °C L/D for batch A; and (ii) from 10 °C L/D to 20 °C L/D for batches B-E.

Germination was recorded every 2-3 days in a darkroom, under green safelight and germinated seeds were discarded after each count. The criterion of germination was visible radicle protrusion. Germination experiments were completed when no additional seeds germinated for 2 weeks (Skordilis & Thanos 1997). When germination tests were completed, cut tests were performed on the non-germinated seeds and final germination percentages were corrected for sound seeds. The germination rate ( $T_{50}$ ) of untreated seeds, expressed as time to 50% of final germination, was calculated following Skourti & Thanos (2015).

**Cotyledons**

Following each germination test, germinants (Fig. 2c) were developed in a plant growth chamber for a short period (2-3 weeks at 15 °C) and then transplanted outdoors in the nursery, in planting trays (1 part humus : 1 part peat : 1 part perlite). The number of cotyledons per individual was counted in a total of 555 seedlings (81, 85, 36, 125, and 228 seedlings from batches A to E, respectively).

**Present and future climatic conditions**

Climatic data were extracted from the WorldClim datasets (WorldClim 2020), in order to investigate the possible effects of climate change on *P. heldreichii* natural populations. For the present period, the climate data for 1984-2014 were used to con-

struct the Walter & Lieth (1967) ombrothermic diagrams for both study areas, produced using the “climatol” R package with minor changes on its base code. For the future climatic conditions, the CNRM-ESM2-1 earth data model (Séférian et al. 2019) for the period 2081-2100, was used along with two shared socio-economic pathways (SSP245 and SSP585 – Riahi et al. 2017). For both cases the spatial resolution used was 2.5 min (~21 km<sup>2</sup>).

**Statistical analysis**

Germination percentages were arcsine transformed prior to analysis. Differences among light conditions in various temperatures in untreated seeds and durations of chilling pretreatment for each seed batch were tested by one-way analysis of variance (ANOVA), followed by either Tukey’s multiple comparisons test, or the non-parametric Kruskal-Wallis test, depending on whether the ANOVA assumptions were met (normal distribution and homogeneity of variance). All analyses were performed using the software package SPSS Statistics® v. 23 (IBM, Armonk, NY, USA).

Pearson’s Chi-squared test of goodness of fit for normality was performed for the number of cotyledons (confidence level 95%), using R v. 3.6.3 Programming Language (R Development Core Team 2005). Throughout the study, the ± values refer to the standard errors of the mean (SE).

Furthermore, a model of Quasi-Poisson family and log link function were fitted to predict germination (g) with chilling (C), temperature (T), light (L), transfer (t) and location (S) (formula:  $g = C+T+L+t+S$ ). The model’s intercept is at -3.81 ( $t = -16.75$ ,  $p < 0.001$ ).

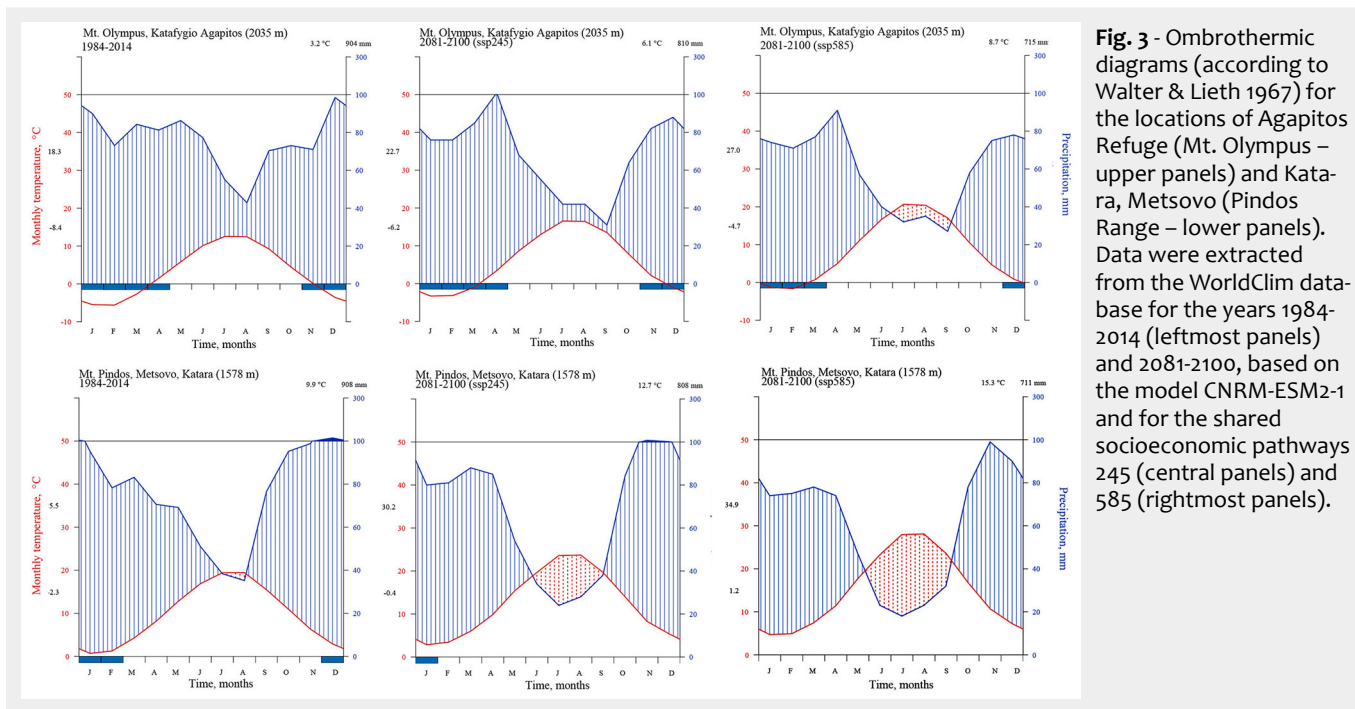
**Results**

The average mass of freshly collected cones varied among batches from 11.8 ± 0.6 to 15.9 ± 0.7 g, with minimum and maximum values of individual cones at 7.5 and 24.3 g, respectively. The mean number of seeds per cone ranged between 60.1 and 78.7 seeds, depending on the collection year and provenance. Seed mass (average per cone) ranged from 22.6 ± 0.9 to 31.1 ± 1.3 mg (Tab. 1) with an overall mean 26.7 ± 0.8 mg. The average contribution (%) of seeds to the total cone mass varied among the 5 batches from 11.4% to 12.1%, with marginal values (of individual cones) at 4.7%

**Tab. 1** - Cone mass and seed characteristics of *Pinus heldreichii* according to Greek provenances and date of collection.

| Provenance                   | Altitude (m a.s.l.) | Date of collection | Cone mass (g) (min-max)       | n  | Seeds per cone (min-max) | Seed mass (mg) (min-max)      | Seed/cone mass (%) (min-max) |
|------------------------------|---------------------|--------------------|-------------------------------|----|--------------------------|-------------------------------|------------------------------|
| Pindos Range (Metsovo)       | 1400                | 21/10/2011         | 15.89 ± 0.72<br>(9.65-24.35)  | 20 | 61.05 ± 3.33<br>(36-84)  | 31.13 ± 1.34<br>(17.13-39.11) | 12.10 ± 0.75<br>(6.39-17.52) |
|                              |                     | 11/10/2012         | 11.80 ± 0.56<br>(7.50-16.90)  | 30 | 60.07 ± 3.05<br>(20-85)  | 23.72 ± 1.63<br>(8.16-50.00)  | 11.59 ± 0.57<br>(4.94-18.00) |
| Mt Olympus (Agapitos Refuge) | 2200                | 15/10/2012         | 15.59 ± 0.63<br>(10.10-24.00) | 32 | 78.66 ± 3.89<br>(24-130) | 22.62 ± 0.85<br>(12.35-32.39) | 11.37 ± 0.53<br>(4.74-16.59) |





**Fig. 3** - Ombrothermic diagrams (according to Walter & Lieth 1967) for the locations of Agapitos Refuge (Mt. Olympus – upper panels) and Katara, Metsovo (Pindos Range – lower panels). Data were extracted from the WorldClim database for the years 1984-2014 (leftmost panels) and 2081-2100, based on the model CNRM-ESM2-1 and for the shared socioeconomic pathways 245 (central panels) and 585 (rightmost panels).

**Tab. 2** - Generalized linear regression model (GLM) predicting the influence of variables on *Pinus heldreichii* seed germination.

| Predictors                | Incidence Rate Ratios | Confidence Intervals (CI) | p-value |
|---------------------------|-----------------------|---------------------------|---------|
| (Intercept)               | 0.02                  | 0.01 - 0.03               | <0.001  |
| chilling                  | 1.03                  | 1.01 - 1.04               | <0.001  |
| temperature               | 1.03                  | 1.02 - 1.05               | <0.001  |
| light                     | 1.44                  | 1.34 - 1.54               | <0.001  |
| transfer                  | 1.34                  | 1.23 - 1.46               | <0.001  |
| provenance                | 1.49                  | 1.33 - 1.66               | <0.001  |
| R <sup>2</sup> Nagelkerke | 0.446                 |                           |         |

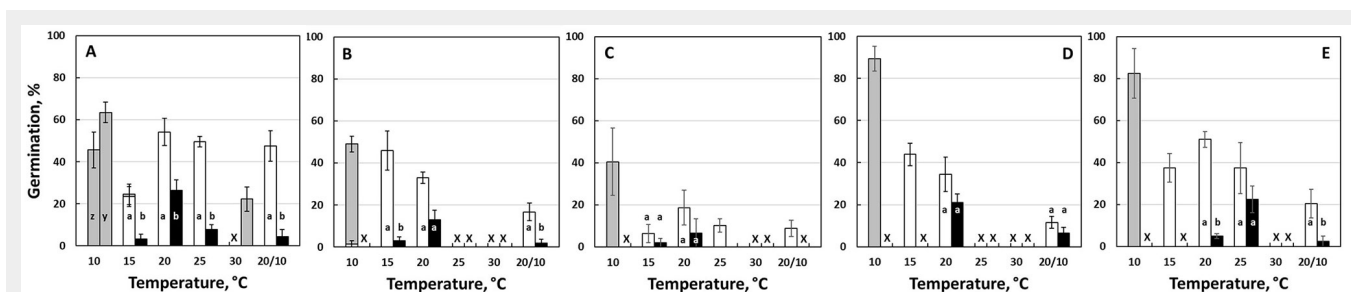
batches D and E, respectively.

According to the presented climate change scenarios, the under-study populations may face increased temperatures and prolonged periods of drought (Fig. 3). Seed germination of untreated seeds (Fig. 4) was promoted by white light, with best results obtained at constant temperatures, 15 or 20 °C (12:12 h L/D) with some exceptions (e.g., in batch C). However, final germination of untreated seeds under light was low or moderate (<55%) for all batches and treatments at 15 or 20 °C; at lower or higher temperatures (10 and 30 °C) no germination was recorded. Final germination of cold stratified seeds under 15 and 20 °C, but not under 10 °C, was significantly improved both in L/D and D (Fig. 5), whereas a 45-day chilling pretreatment (in some cases even a 30-day-long period) was sufficient for full germination under light/dark conditions at 15 and 20 °C.

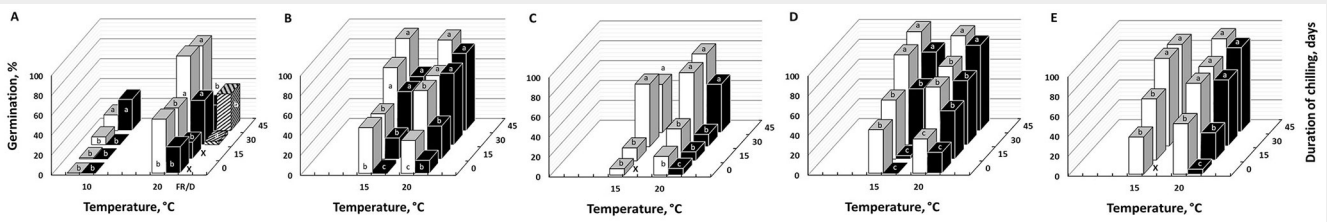
The effect of (i) chilling period (t = 17.65, p

and 18.0% (min and max). The mean number of cotyledons per seedling was 7.25 ± 0.04 (n = 555), the absolute values ranged from 5 to 9 and, although non-continuous variables, its frequency approximates the normal distribution (p-value = 0.8921). The

overall mean percentage (%) of empty seeds, estimated from post-germination cut-tests, was 10.2%, 24.4% and 51.6% for Pindos Range batches A, B and C, respectively. Similarly, for Mt. Olympus, the relevant percentages were 12.1% and 19.9% for



**Fig. 4** - Final germination in untreated (non-stratified) *Pinus heldreichii* seeds, tested over a range of constant (10, 15, 20, 25 and 30 °C) and alternating temperatures (20/10°C), in the light (12 h photoperiod, white bars) or in continuous darkness (black bars). Different small-case letter labels indicate significantly different values between light and dark treatments within each temperature (p < 0.05). Results from Pindos Range collections are shown in graphs A (collection year 2011), B (2012) and C (2013), while Mt. Olympus collections appear in graphs D (2012) and E (2013). Non germinated (untreated) seeds of the non-stratified treatment were subsequently transferred as follows: (i) from 10 (L/D and D), 15 (L/D) and 30 °C (D) to 20/10 °C L/D for batch A; and (ii) from 10 °C L/D to 20 °C L/D for batches B-E (results are presented by grey bars). The “x” mark indicates lack of experimental data in the respective germination conditions.



**Fig. 5** - Effect of various chilling durations (0, 15, 30 or 45 days) on final germination of *Pinus heldreichii* seeds at 10, 15 and 20 °C, under white light (12 h photoperiod, white bars), dark (black bars) and Far Red light (spotted bars). Different small-case letter labels indicate statistically significant differences among germination values obtained by different chilling durations within each temperature ( $p < 0.05$ ). Results from Pindos Range collections are in graphs A (2011), B (2012) and C (2013), while collections from Mt. Olympus appear in graphs D (2012) and E (2013). The “x” mark indicates lack of experimental data in the respective germination conditions.

$< 0.001$ ), (ii) temperature ( $t = 4.02$ ,  $p < 0.001$ ), (iii) light ( $t = 10.22$ ,  $p < 0.001$ ), (iv) transfer to more favorable temperatures and light ( $t = 6.54$ ,  $p < 0.001$ ), and (v) provenance ( $t = 7.08$ ,  $p < 0.001$ ) were all statistically significant and positive, as shown by the GLM model (Tab. 2).

All non-germinated seeds from the lowest, constant temperature (10 °C) experiments, both experiencing alternating light/dark conditions and continuous darkness, did not germinate after 5-6 weeks at 10 °C; after being transferred to a higher temperature (20 or 20/10 °C) in light/dark (grey bars in Fig. 4), they germinated to a considerable degree (41-89%).

The germination rate of untreated seeds was rather low;  $T_{50}$  ranged between 9 and 14 days in light germination experiments and 13 to 22 days under continuous darkness. A remarkable reduction of germination rate was achieved following stratification ( $T_{50}$  is lower than one week in seeds prechilled for 45 days). The time courses of *P. heldreichii* seed germination, for both untreated and stratified seeds, at 15 °C, in L/D and D presented in Fig. 6a and Fig. 6b, respectively, show a clear and considerable enhancement of germinability by light and cold stratification.

### Discussion

Natural regeneration of *Pinus heldreichii* depends exclusively on seeds, therefore seed germination data, concerning temperature dependence as well as light and/or stratification requirements, are of paramount importance for the biology and conservation of the species, especially when climate change is taken into consideration.

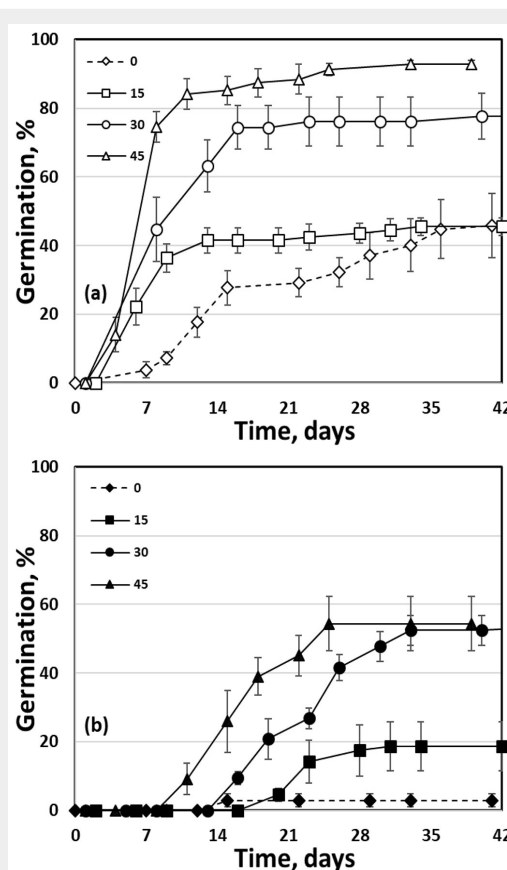
On the basis of both morphological and molecular evidence (Jin et al. 2021), *P. heldreichii* belongs to the subsection *Pinaster*, section *Pinus*, subgenus *Pinus* (diploxylon or hard pines), together with the typical, low-altitude Mediterranean pines *P. halepensis*, *P. brutia*, *P. pinaster* and *P. pinea* and the closely interrelated pair of *P. canariensis* and *P. roxburghii* (Canary Islands and Himalayas, respectively). Average seed mass values obtained in this study for *P. heldreichii* (22.6 up to 31.1 mg), were between those reported for low-altitude Mediterranean pines *P. halepensis* (20 mg) and *P.*

*brutia* (51 mg), although they might vary among collections and years and might be related to seed germination and seedling performance (Thanos 2000, Daskalou & Thanos 2010). The cotyledon number per seedling, an indirect measure of both seed mass and initial photosynthetic capacity, significantly affects seedling survival (Daskalou & Thanos 2010). Mean cotyledon number in *P. heldreichii* seedlings (7.25) was quite similar with those reported for *P. halepensis* (7.4 – Daskalou & Thanos 2010), *P. brutia* (8.4 – Thanos 2000) and *P. canariensis* (8.2), with higher values obtained in heavier *P. pinea* (11.1) and *P. roxburghii* (11.2) seeds (Saeed 2000).

Masting has not been solidly proven for *P. heldreichii*, although there is some circumstantial evidence such as the irregularity of seed production between years (Sto-

jicic et al. 2004) as well as variable germination percentages (Stojičić et al. 2008). High proportions (about 50%) of empty seeds in the seed batches of the Pindos Range provenance (C) shown in the present study is an additional indication of masting occurrence.

Without any pretreatment, seeds of *P. heldreichii* from both populations are observed to germinate to low or moderate levels ( $< 55\%$ ), at 15-25 °C under alternating light/dark conditions. The inability of germination in darkness, mentioned previously for Greek populations (Skordilis & Thanos 1997), seems to be significantly reduced by white light at all temperatures tested. At low and high temperatures (10 and 30 °C), germination was fully suppressed in the dark; however, upon transfer of the non germinated seeds from darkness to light,



**Fig. 6** - Time course of *Pinus heldreichii* seed germination at 15 °C, (a) in alternating light/dark conditions (12 h photoperiod), and (b) in continuous darkness, following cold stratification (at 2-4 °C in darkness) for 0, 15, 30 or 45 days. Seeds were collected from the Pindos Range provenance in 2012.

significant levels of germination were recorded. It must be pointed out that higher germination percentages were also achieved upon transfer of non-germinated seeds from low to higher temperatures, *i.e.*, from 10 to 20 °C, indicating a “chilling” pretreatment requirement (Borghetti et al. 1986).

In addition to white light, germination of *P. heldreichii* seeds was promoted by cold stratification as well (reported also by Borghetti et al. 1989). Cold stratification treatments have been traditionally used to break physiological dormancy (PD) in tree seeds, while if untreated, these seeds require more than 4 weeks of incubation prior to the initiation of germination (Baskin & Baskin 2014). In accordance with older studies (Borghetti et al. 1989, Giannini & Bellari 1995, Baskin & Baskin 2014), a long period (70 days) of cold stratification is required to break PD in *P. heldreichii* seeds. Our results suggest that cold stratification is not an absolute requirement for seed germination, although a chilling period of 30-45 days enhances both final percentage and rate of germination, in agreement with the International Rules for Seed Testing (ISTA 2007). Therefore, according to an ecophysiological interpretation of our germination experiments, under the current climatic conditions, *P. heldreichii* seeds, following their autumn dispersal from cones, are subjected to cold stratification (under winter conditions) that will lead to a late spring-early summer germination, when temperatures are favourable (>15 °C) for seedling establishment, growth and survival. These results are in accordance to the earlier findings for the Italian Heldreich's pine populations (Borghetti et al. 1986, 1989).

According to the various climate change scenarios, the Mediterranean region will be particularly vulnerable to an anticipated temperature increase and precipitation decrease, likely resulting in amplified drought episodes and extreme temperatures, with cold resistant conifer habitats of high mountains being quite sensitive (Xystrakis et al. 2006).

Taking into account the germinability of a proportion of seeds without prior chilling stratification, we may expect that the rising temperature, according to the two SSPs scenarios, may not affect significantly the seed germination and the subsequent initiation of seedling establishment. According to the climatic predictions, a high possibility of an extensive period of drought during early summer to late autumn (3-4 months) is obvious, especially in the case of the Pindos Range (Metsovo), as presented in the ombrothermic graphs for both the study areas. The probable increased drought season may have a significant effect on *P. heldreichii* seedling recruitment by increasing the mortality rate. The predicted change of both temperature and rainfall appear more extreme in the case of the Pindos Range. In the case of Mt. Olympus, where the population is growing at a

higher elevation, we expect (at least for the milder SSP scenario) no significant drought period in the summer. Based on the above assumptions, the population in Pindos Range will face harsher climatic conditions, which may jeopardize its survival in the next century.

Finally, *P. heldreichii*, a thin-barked and non-serotinous pine, is not adapted to fire (Ne'eman & Arianoutsou 2021); the species does not survive even after swift surface fires, and shows a very limited post-fire regeneration, however it may invade burned areas from neighbouring unburned clusters. So far, no specific protocol for post-fire management has been proposed (Arianoutsou et al. 2012).

## Conclusion

In the face of climate change, with predicted higher temperatures, prolonged drought periods and increased wildfire risk, *P. heldreichii* populations and habitats in Greece may be threatened, rendering particularly vulnerable populations established at lower altitudes; *ex situ* (*e.g.*, germplasm conservation) and *in situ* measures (*e.g.*, selection for drought tolerance) are recommended for the future conservation of the species.

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## Author contributions

Conceived and designed the experiments: END and CAT; performed the experiments: END; drafting of the manuscript and analyzed the data: END, KK and SO; contributing to materials/analysis tools: KK and SO; critical revision of the manuscript: CAT.

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