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RESEARCH

From cone to seedling: Reproductive biology of *Juniperus drupacea* at its westernmost distribution limit

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Abstract

Juniperus drupacea is a Tertiary relict, dioecious tree, rare in Europe, found exclusively in Greece; its primary population located in Parnon Mt. (Peloponnese). In Europe, the species is considered as Endangered by the IUCN Red List, facing primary threats such as sapling grazing, timber overexploitation, fire and climate change. Data on cone/seed morphometric traits and seed viability/germination are analyzed with a focus on the implications for restoration and conservation efforts. Cones were collected in October—November of 2020–2023. The average cone mass is 3539.0 ± 95.5 mg, with each cone containing 1–5 viable seeds (mean of 2.3 seeds/cone); the average seed mass is 33.9 ± 2.6 mg. Seed germination was studied under constant (10, 15, 20 °C) and alternating (20/10, 25/15 °C) temperatures, after eight pre-chilling regimes (0, 4, 8, 12, 16, 20, 24 and 32 weeks) at 5 °C, both in continuous darkness or under light/dark (12 h photoperiod); the effect of GA_3, with 16 weeks of pre-chilling or without pretreatment was also tested. Viability of seeds stored in a Seed Bank was assessed using the tetrazolium test (TTZ). The optimal seed germination was observed at 15 °C, while both pre-chilling (up to 16 w) and GA_3 treatment lead only to an increased germination rate. Seeds are light-indifferent, exhibit orthodox storage behavior, remaining viable for long periods (> 15 years). Seed storage within cones is more effective than in the form of exposed, 'naked' seeds. Based on our field observations, seedlings exhibit a high mortality rate during their first year.

Keywords Cone morphology · Conservation · Germination ecophysiology · Seed morphology · Seed viability · Syrian juniper

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Introduction

Juniperus (Cupressaceae) stands out as one of the most diverse conifer genera, comprising approximately 75 species (Adams 2014; Dörken 2019). Widely distributed in temperate and subtropical regions of the Northern Hemisphere, extending southward to tropical Africa (Bonner 2008), the genus includes around 20 taxa found in Europe, the Canary Islands, Azores, Asia Minor, and Africa (Adams 2014). Within *Juniperus,* one of the three monophyletic sections is *Caryocedrus* Endl. (Adams 2014; Sobierajska et al. 2016), which is uniquely represented by a single species, *Juniperus drupacea* Labill., found on the mountains along the Mediterranean Sea in South-West Asia and in South-East Europe (Browicz & Zielinski 1982; Dörken 2019; Boratynski et al. 2023) (Fig. 1).

Juniperus drupacea (Syrian juniper) is a Tertiary relict dioecious tree species (Fig. 2) characterized by distinct male and female individuals. Molecular and biochemical characteristics (Boratynski et al. 2023) and unique morphological features, specifically the unusually large seed cones (up to 3 cm in diameter) with three fused scales, encompassing three ovules, forming a hazelnut-like "stone" at maturity, set this species apart from its congeners. Additionally, the male reproductive structures (cones-microstrobili), display an inflorescence-like arrangement (Dörken 2019), a feature absent in other *Junipers* (Boratynski et al. 2023).

Juniperus drupacea global geographical distribution is relatively limited and fragmented, mostly in SW Turkey, Western Syria, Israel and Lebanon (Boratynski et al. 1992). In Europe, its presence is restricted to SE Peloponnese, Greece, specifically on Mt. Parnon and a small area of Mt. Taygetos, where it forms scattered patches (Tan & Iatrou 2001; Bergmeier 2002; Dimopoulos et al. 2013). The *J. drupacea* populations in Mt. Parnon are of particular ecological interest (Daskalakou et al. 2022a, b), as they represent more than 95% of the species distribution area in Greece (Constantinidis & Kalpoutzakis 2015). Mt. Parnon juniper forests grow on calcium rich soils, developed from different limestone rocks, at an altitude of 700–1600 m a.s.l., and form either pure or mixed stands with black pine (*Pinus nigra* J.F.Arnold subsp. *nigra*) and the Greek fir (*Abies cephalonica* Loudon) (Bergmeier 2002; Maerki & Frankis 2015).

Globally, *J. drupacea* is classified as Least Concern (LC) by the International Union for the Conservation of Nature (IUCN) (Gardner 2013, 2017). However, in Europe, it is considered Endangered (EN) (Rivers et al. 2019), as it is represented solely by the Greek populations, and it is protected at the national level by Presidential Decree 67/1981. Additionally, it plays a vital role as the main structural plant in the subtype 42.A5 (Syrian juniper woods) of the priority habitat "9560*—Endemic forests with *Juniperus* spp." according to Directive 92/43/EEC (Anonymous 2013). The broader area of Mt. Parnon has been included (GR2520006) in the European Natura 2000 Network of protected areas, underscoring the importance of conserving these natural juniper stands.

The species faces threats from climate change, and specific conservation strategies are recommended for preserving its genetic and morphological diversity (Walas et al. 2019). The genetic and morphological differentiation among the disjunct populations of the species, attributed to the long isolation between the mountainous populations of Southeast Asia and the European ones, separated by the Aegean Sea and an estimated distance of almost 800 km, further highlights the challenges posed by the species' fragmented natural distribution (Tan et al. 1999; Sobierajska et al. 2016).

Given the species' fragmented natural distribution and the predicted reduction in its global range (Walas et al. 2019; Kokkoris et al. 2023), it may face significant declines

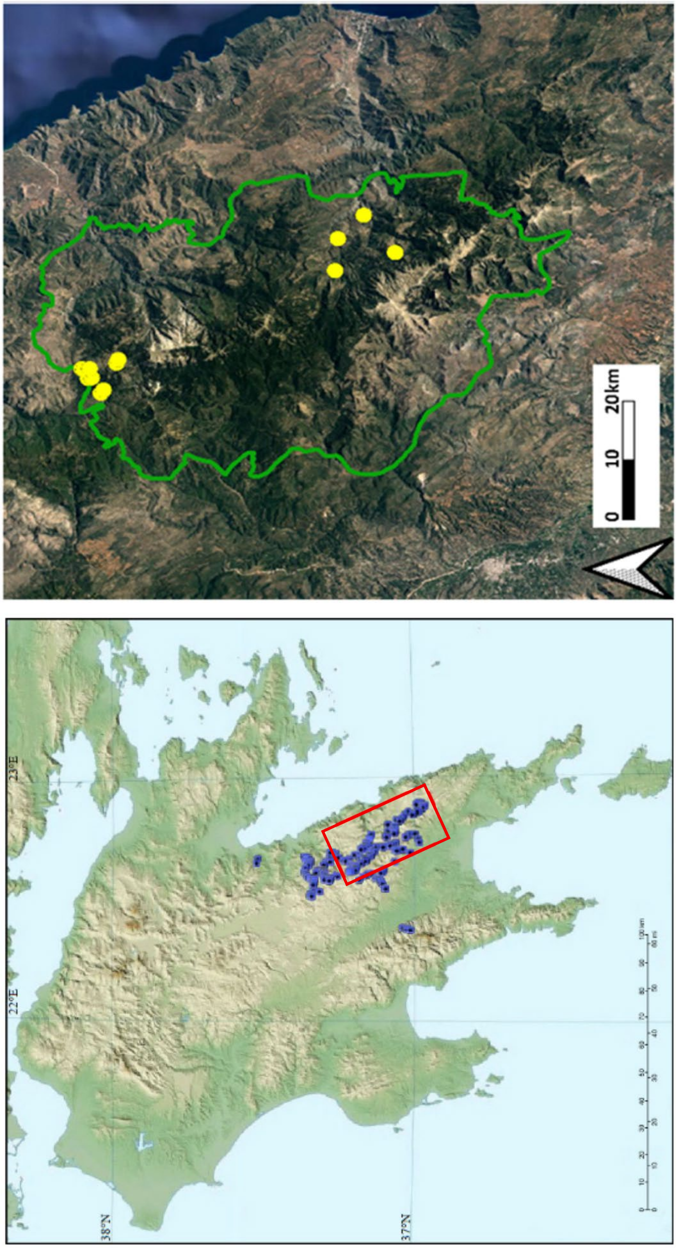

Fig. 1 *Juniperus drupacea* distribution range in the Peloponnese, Greece (adapted from Maerki & Frankis 2015, left); map with *Juniperus drupacea* monitoring plots in the northern and southern part of Mt. Parnon (right) within the boundaries of the NATURA 2000 site (green line)

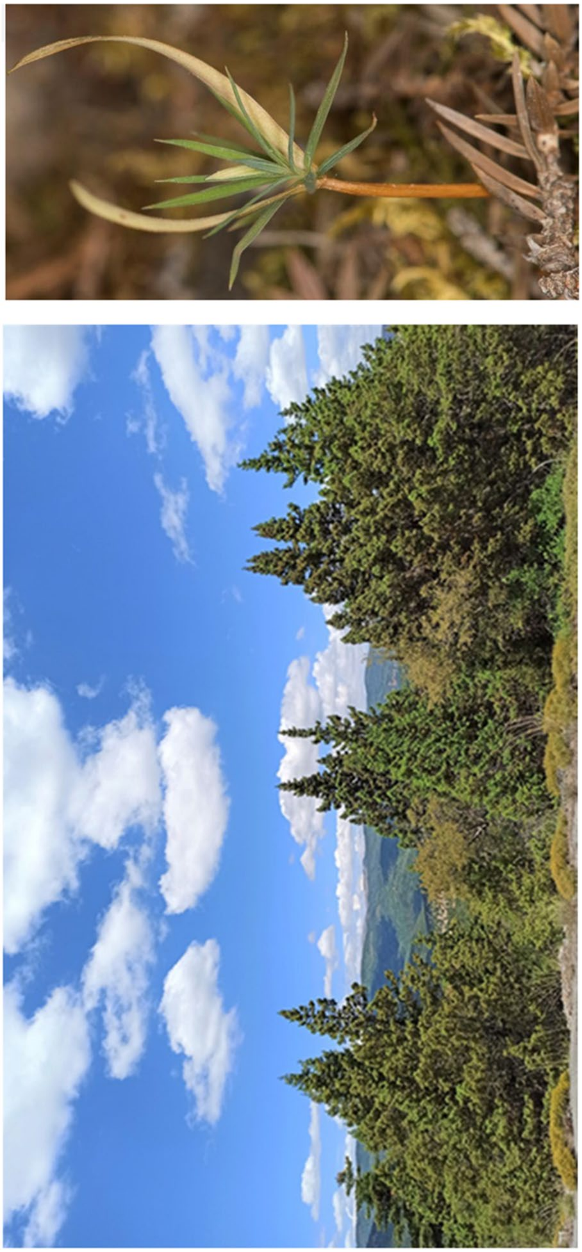

Fig. 2 *Juniperus drupacea* individuals from the populations of Mt. Parnon (left); new seedlings, monitored in the field, at the cotyledon stage (right)

in the coming years. Recent studies on Greek populations (e.g. Avramidou et al. 2023; Ioannidis et al. 2023; Solomou et al. 2023; Korakaki et al. 2024) report low genetic diversity in the Mt. Parnon, increasing drought stress, and difficulties on in vitro clonal propagation. Establishing conservation targets and developing accessible seed-based propagation protocols for long-term in situ and ex situ conservation will support the species' future survival.

The present study aims to investigate *J. drupacea* seed and seedling biology, with the use of germination tests performed under various temperatures, chilling treatments and light regimes. A 4-year (2020–2023) study of seed germinability was carried out on cones and seeds collected from Mt. Parnon. Given the limited research on Syrian juniper seed germination (Yavuz & Yilmaz 2017; Yucedag et al. 2021), data obtained in this study could contribute valuable insights into its germination mechanisms under natural conditions, seedling establishment and natural regeneration trends of 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

The study was conducted over a 4-year period (2020–2023) in the mountainous Parnon range in SE Peloponnese, Greece. The research focused on investigating the *J. drupacea* seed biology and germination ecophysiology, including the monitoring of natural regeneration (2021–2022), seedling emergence, density and survival in the field across 13 monitoring plots (Fig. 1).

Natural regeneration monitoring

Seedling dynamics were assessed on Mt. Parnon through the establishment of 13 permanent transects, one in each monitoring plot, with an area of 20 m^2 (10×2 m each). These transects were positioned at the center of the 13 monitoring plots within the Syrian juniper forest (Daskalakou et al. 2022a, b), minimizing edge effects (Fig. 1). Each transect was permanently marked in the field using four metal pins, one at each corner.

A census of *J. drupacea* seedling emergence and density (seedlings m^{-2}) was conducted within the transects twice per year: once in late spring-early summer (May–June) and again in the fall (September–October), over a 2-year monitoring period (2021–2022). During this period, the absolute number and age of saplings were also monitored in each transect. Seedling and sapling age in the field was determined based on literature (e.g., Boratynski et al. 2023) and observations of seedlings, that were produced by the germination experiments, grown and monitored in the laboratory for at least one year. Two major categories of *J. drupacea* seedlings/saplings were identified in the field: a) young, recently germinated and early emerged, new seedlings, still at the cotyledon stage (< 1 year of age) and b) saplings older than 1–3 years. For each monitoring period, juniper seedling emergence and seedling-sapling density per m^2 were subsequently estimated.

Cone collections, biometric characteristics and seed processing

Cone collections of *J. drupacea* were carried out each fall (October–November) throughout the study period. Approximately 30 mature cones per tree, on average, were gathered from the base of about 10 randomly selected trees within or near the 13 established monitoring plots. This procedure was performed for both the northern and southern populations of Mt. Parnon for every maturation and collection year from 2020 to 2023 (4-year sampling period).

Cones were stored at room temperature (approximately 20 °C) for at least two weeks before undergoing cone and seed processing. Seed extraction was performed manually, using scissors to remove the fleshy parts, a combination of a hammer and tongs to crush the woody sections of the cones, and forceps to select and collect intact seeds.

Over a 3-year period (2021–2023), a randomized selection of Syrian juniper cones (n = 60–220) subjected to a detailed morphometric analysis, including the quantification of seed number and mass. Cone diameter at its longest circumference point was measured, and cone mass was determined using a high-precision analytical balance (OHAUS PX124—S/N C049640285, accuracy 0.1 mg). Each cone was carefully opened, and the mature seeds were counted. The mass of each single seed was measured at moisture content of 5–6%, based on the low constant temperature method (ISTA 2007) with two samples of 50 seeds. The number of seeds varied based on the number of available cones (see Table 1); specifically for year 2021 the mass of 739 seeds was measured, for 2022 and 2023 the number of seeds measured was 457 and 354, respectively.

In addition to the procedures described, an extensive analysis of 1029 cones for the years 2020 and 2021 was conducted to assess the number of seed slots, mature seeds, and aborted seeds within the cones.

Seed germination experiments

Seed germination tests, consisting of 5 Petri dishes with 20 seeds, extracted from mature cones, for each treatment, were conducted in temperature-controlled plant growth chambers (BINDER KBW 240, with a fluorescent [Fluora growth lamp] light source of 7.500 lx, Germany, and HI-POINT, Taiwan, with a LED Z8 light source of 10,000 lx). The chambers maintained a constant temperature within ± 0.5 °C. Two light regimes were employed: continuous darkness (D), with petri dishes placed in light-proof metal containers, and alternating light/dark conditions (L/D) with a photoperiod of 12 h light and 12 h darkness. Seed

Table 1 Cone and seed morphometric traits, mean values and standard errors of the mean, for three *Juniperus drupacea* seed lots (2021–2023)

Year	Sample size	Cone diameter (mm)	Cone mass (mg)	Seed number per cone	Seed mass (mg)
2021	220	18.2 ± 0.2^b	2949.0 ± 72.6^c	2.2 ± 0.1	31.6 ± 0.6
2022	60	18.4 ± 0.3^b	3590.4 ± 142.5^b	2.4 ± 0.1	33.5 ± 5.2
2023	204	19.3 ± 0.1^a	4079.0 ± 71.41^a	2.6 ± 0.1	36.8 ± 1.9

Letters indicate statistically significant differences germination was studied under two temperatures (15 and 20 °C) and across various pre-chilling regimes (Table 2).

For the seed lot of the first year (2020), five different pre-chilling durations were examined (0, 4, 8, 12, and 16 weeks at 5 °C), while the subsequent collection year (2021) involved four durations (20, 24, and 32 weeks at 5 °C). For the third collection year (2022), two pre-chilling regimes (0 and 16 weeks at 5 °C) were tested, with and without the application of 1000 ppm GA3. Lastly, for the fourth year (2023) the effect of 10 °C and of alternating temperatures 20/10 °C and 25/15 °C was tested without pre-chilling of seeds. Following the cold stratification period, seeds were transferred to a temperature of 15 °C in continuous darkness. In the case of the 16-week pre-chilling treatment, germination was studied both under alternating light/dark conditions (15 °C) and continuous darkness at 20 °C for the 2020 collections. Meanwhile, for the 2022 and 2023 collection, all experiments were conducted under alternating light/dark conditions.

As a follow up experiment, the ungerminated seeds from 0, 4, 8, 12 and 16 w (year of collection 2020) of pre-chilling treatment were transferred back to the chilling conditions (at 5 °C). This involved: a) an additional 28 and 24 weeks for the seeds initially subjected to 4 and 8 weeks of chilling, respectively, b) an extra 16 weeks for the control group (0 weeks of initial chilling), and c) an additional 12 weeks for the seeds initially exposed to 16 weeks of chilling at both 15 °C and 20 °C. Notably, the 16-week alternating light/dark experiment concluded without undergoing a second chilling period. For the experiments with the collections from 2020 and 2021, we imbibed the seeds on Whatman filter paper, while for the experiments of 2022 and 2023 water agar (6 g of agar/1 L of water) was used instead (see Table 2). When germination tests were completed, cut tests were performed on the non-germinated seeds and final germination percentages were corrected for sound seeds.

Seed viability

A total of eight collections of the species (2000–2023), including four from the present study and four stored in the National and Kapodistrian University of Athens Seed Bank, were used to assess seed viability. A sample of 50 seeds from each collection was evaluated using the biochemical Topographical Tetrazolium Test (TTZ), a staining method recommended for *Juniperus* spp. (ISTA 2007). Viability testing was conducted in 2022 for collections up to that year and in 2023 for the next year collection (2023). The 2021 collection underwent testing twice: once in the year of collection and again one year after the initial test. Discoloration of seeds stored in room conditions was recorded and was attributed to potential oxidation of the seeds. The different storage conditions and durations can be found in Table 3.

Germination results were adjusted based on the findings of the TTZ tests and the final cut test performed on seeds that failed to germinate by the end of the experiments.

Statistical analysis

Given the non-normal nature of the datasets in this study, the Kruskal–Wallis test was employed to explore differences among the germination, cone and seed morphometry datasets. For pairwise comparisons, the post-hoc Dunn test was applied. For the sapling densities between monitoring years we used the Wilcoxon signed-ranked test. All statistical analyses and graphical representations were conducted using the R programming language version 3.6.3 (R Development Core Team 2005).

Results

Seedling emergence, density and survival

Syrian juniper seed germination and seedling emergence, under natural conditions, usually occurs in a spring massive 'wave', that could start from early spring (April) and last for a few months until summer (June). The absolute values of *J. drupacea* seedlings in all transects monitored in spring 2021 were 58 (0.26 ± 0.08 seedlings m^{-2}) and only 14 (0.06 ± 0.03 seedlings m^{-2}) in the following spring 2022 ($n = 72$ new seedlings overall). The absolute values for older saplings (> 1–3 years of age) were 47 (0.20 ± 0.04 saplings m^{-2}) in spring 2021, and 79 (0,37 ± 0,11 per m^2) in the next spring. During the whole study period, the seedling and sapling density consistently remained below 0.5 per m^2 without a statistical significance (p-value = 0.23) between the years of monitoring.

Cone and seed traits

For the years 2021–2023, the morphometric details of the cones (mass and diameter), seeds (number of mature seeds per cone) and seed mass are provided in Table 1. The number of *J. drupacea* seeds per cone varies from 1 to 5 mature seeds, referred to as seed slots in the cone (Fig. 3). Cones with 1 and 4 seeds were the least common, constituting 2% and 1% of the studied batch, respectively. Over the 3-year study period, only one cone with 5 seed slots was identified. The most prevalent cases were cones containing 2 (23%) and 3 (60.5%) seeds. Based on the total number of cones studied in the initial two years ($n = 1029$), 13.5% were found to contain solely underdeveloped seeds, while the remainder held both fully matured seeds and partially underdeveloped seeds.

Statistical differences were observed in the morphometric characteristics of *J. drupacea* diaspores. Regarding the cones, significant differences were noted between the years 2021–2023 (p-value ≈ 0) and 2022–2023 (p-value = 0.009) in terms of diameter, while significant differences across all years were observed for average cone mass. Cone diameter is not significantly correlated to the included seed number ($p_{\text{Kruskal-Walis}} = 0.54$). Cone mass is significantly correlated to the included number of seeds ($p_{\text{Kruskal-Walis}} < 0.001$) but based on the post-hoc Dunn test significant differences appeared only between cones with aborted and 2 seeds. Conversely, no differences were noted in average seed mass for any of the years, with significant differences only observed in the number of mature seeds per cone for the years 2021 and 2023.

Seed germination

The germination outcomes for the years 2020, 2021 and 2023 are presented in Table 2. *J. drupacea* exhibited germination under both dark (46.7%) and light (38.9%) conditions, with relatively minor differences in the final germination percentages between the two lighting regimes. Germination could commence at low temperatures (5 °C) following an extended chilling period (> 20 weeks). Based on the first year of testing, germination

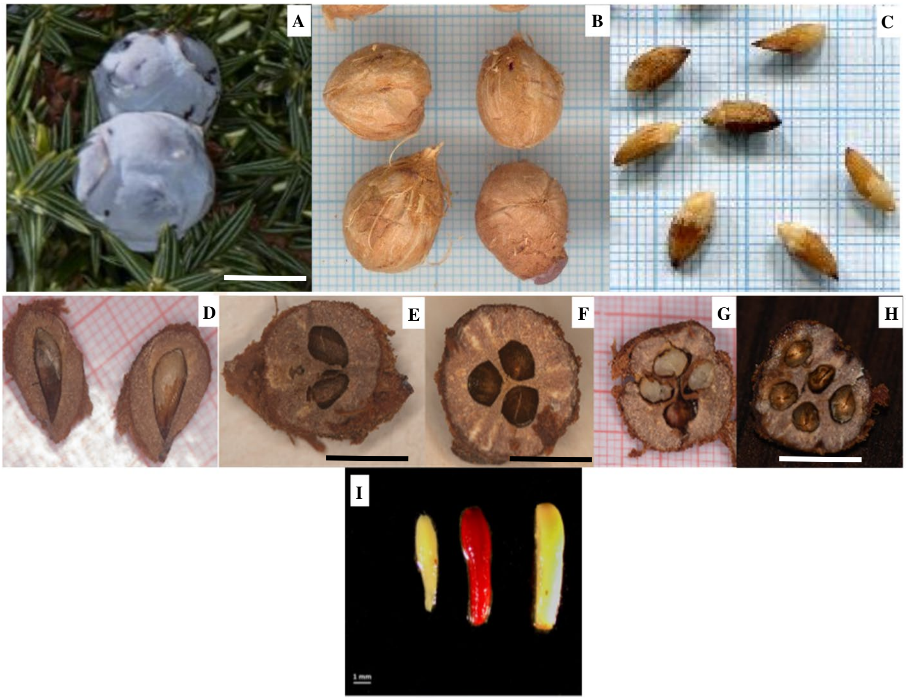

Fig. 3 *Juniperus drupacea* mature cones with their leathery covering (**A**), cones without the fleshy parts (**B**) and seeds (**C**). Cones may include one (**D**), two (**E**), three (**F**), four (**G**) and five (**H**) seed cavities/seed slots on their woody interior. Embryos (**I**) after TTZ viability staining, recommended for *Juniperus* spp. (ISTA 2007): from left to right i) underdeveloped dead embryo, ii) viable embryo and iii) fully developed dead embryo. White and black bars: 1 cm

Table 2 Final *Juniperus drupacea* seed germination results, mean values and standard errors of the mean, for the three seed lots (2020, 2021 and 2023)

Collection (years)	Pre-chilling in D (weeks)	Light conditions[1]	Temperature (°C)	Germination (% ± SE)
2020	0	D	15	28.7 ± 6.7^{c}
	16	D	15	46.7 ± 4.9^{a}
	16	L/D	15	38.9 ± 6.9^{b}
	16	D	20	7.8 ± 2.5^{d}
2021	20	D	15	23.5 ± 3.0
2023	0	L/D	20/10	18.5 ± 11.4^{f}
	0	L/D	25/15	28.8 ± 4.5^{g}

[1]Light conditions: D in continuous darkness and L/D under alternating light/darkness (12/12 h)

Letters indicate statistically significant differences

showed improvement after a chilling period, with 16 weeks identified as the optimal duration for achieving the highest germination (46.7%). Among the other tested chilling periods, results varied significantly, and sowing without pre-treatment (0 weeks of chilling) yielded the second-highest germination (28.7%). For the 2021 experiments the different chilling periods did not present statistically significant results.

In the 2022 experiments (Fig. 4A), agar was used instead of filter paper for germination tests, resulting in significantly improved outcomes, as the infections observed in previous seed collection years and experiments were considerably reduced. The best germination results (95.0 ± 5.0, T_{50} = 62.0 days, were T_{50} is the time in days for 50% of the seeds to germinate (Skourti & Thanos 2015), were obtained without any chilling (Fig. 4A), while the 16-week chilling treatment significantly enhanced only the germination rate (73.7 ± 11.6, T_{50} = 13.0 days). The percentage of dead and infected seeds was consistently high (> 30–44%) in each collection year and germination test, respectively.

The germination experiments for the first two years (2020–2021) were notably prolonged, lasting over 12 months, resulting in a high mortality rate due to extensive fungal development on the sowing paper and subsequent seed transfers to new Petri dishes. In comparison, the experiments conducted in 2022 (Fig. 4A), with the transition from filter paper to water agar for germination tests, demonstrated significant improvements in experiment duration (approximately 7 months instead of over a year). The corrected germination, after the final cut test of the experiments, showed a markedly higher final germination percentage. The highest germination achieved in agar was 95% (see Fig. 4A) without chilling, attained 192 days after sowing. The 10 °C have a slight negative effect on the final germination (79.3 ± 9.4) compared to the 15 °C, while the alternating temperatures resulted in significantly lower final germination (20/10 °C—18.5 ± 11.4 and 25/15 °C—28.8 ± 4.5). The above values referred to corrected final germination taking into account the dead seeds (cut test).

Lastly, the secondary periods of chilling during the first year of experiments (follow-up experiments) did not significantly affect the final germination; the only exception is the experiment without initial pre-chilling, showing an 14% increased final germination, while the rest of the experiments showing small changes 1–5%.

Seed viability

The TTZ tests indicated the presence of viable seeds in the majority of the available collections. The only exceptions were the collections from 2000 and 2013 (Table 3), stored as seeds and as cones with the pericarp, respectively, which exhibited no viable seeds. Similarly, the viability of the 2021 seed lot, stored as seeds, was notably lower, measuring 26.5% during the second TTZ test compared to the initial one (54.0% measured immediately after seed collection). In contrast, the remaining collections, stored inside their cones (with or without the leathery, outer covering), demonstrated high viability during the tests (> 73.4%). However, for the most recent collection of 2023, the viability was found to be lower at 46.0% (Table 3).

Discussion

Greece is a biodiversity hotspot in Europe due to its plant richness and high endemism levels (Georghiou & Delipetrou 2010; Dimopoulos et al. 2013), with the Peloponnese area, hosting *J. drupacea* populations, recognized as one Mediterranean Basin hotspots (Médail & Quézel 1999; Médail & Diadema 2009). Junipers are slow-growing, long-lived evergreen trees or shrubs with high adaptability to arid environments, occurring across habitats

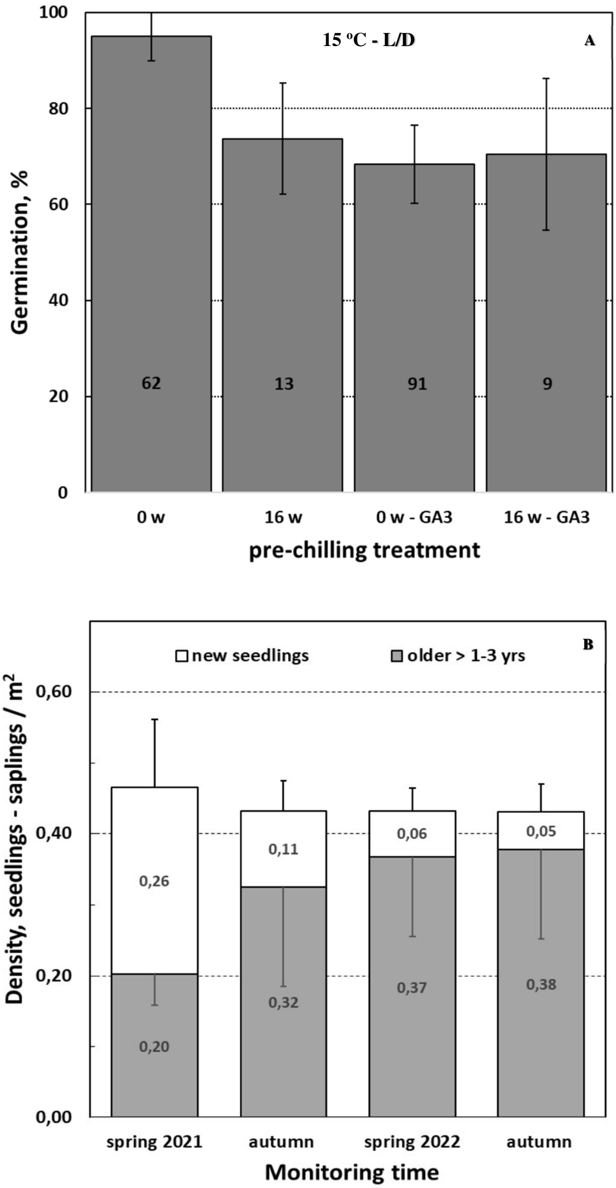

Fig. 4 Final *Juniperus drupacea* seed germination (**A**) for untreated seeds and seeds treated with GA_3 and/or pre-chilled for 0 and 16 weeks (w), in alternating light/dark regime (12/12 h) at 15 °C. Germination percentages were corrected, based on the cut test implemented at the end of the experiments; the period for the 50% of the seeds to germinate (T_{50}, values in days), are shown inside the bars. Experiments correspond to the cone/seed collection of 2022 maturation year (seed lot 2022). Seedling and sapling (at the age of > 1–3 yrs old) density per m^2, measured in 13 monitoring plots in the northern and southern part of Mt. Parnon (**B**)

Table 3 *Juniperus drupacea* seed viability under different storage conditions and periods

Collection (year)	Storage (years)	Specimen state[1]	Storage conditions[2]	Viability (%)
2000	24	seeds	lab	0.0
2009	15	cones*	lab	73.4
2013	11	cones	4 °C	0.0
2013	11	cones	−20 °C	86.0
2020	4	cones	4 °C	90.0
2021	3	seeds	4 °C	26.5
2022	2	cones	lab	91.8
2023	1	cones	4 °C	46.0

[1]Specimen state: The collection was stored: a) seeds: as cleaned seeds, b) cones: as whole cones without any processing and c) cones*: as cones after the removal of the fleshy outer cover

[2]Storage conditions: lab: Laboratory conditions – stored in a box in darkness

from sea level to above the timberline (Adams 2014). This study provides the first assessment, according to our knowledge, of seed biology and seedling dynamics in *J. drupacea* populations on Mt. Parnon, Greece.

Distinct morphological differences in cones and seeds of *J. drupacea* populations in Greece are formerly documented (Sobierajska et al. 2016), with reported smaller cone mass, dimensions, and seed mass. Variations in these morphological features exist between different collection years, adding a temporal dimension to the observed differences.

The TTZ stains, used in this study, reveal notable variability in seed viability. Intriguingly, seeds stored within cones might exhibit a tendency to maintain viability for a longer period compared to those stored as individual seeds, although further investigation is required. This disparity in longevity is possibly linked to the potential oxidation phenomenon, based on the discoloration of the seeds, notably present in the oldest seed lot stored as plain seeds (2000 collection year, 23 yrs of storage). In contrast, seeds extracted from cones (2009 collection, 15 yrs of storage) display minimal signs of oxidation, with the majority retaining viability. While the orthodox seed storage behavior is commonly assumed for many juniper species (Bonner 2008; Rantala-Sykes 2017), the present study marks the first demonstration of such behavior in *J. drupacea*. Viability staining, conducted over multiple collections and spanning 23 yrs of storage under diverse conditions, provides evidence for the species' ability to remain viable both in laboratory settings and in more conventional seed banking conditions at −20 °C. Additionally, the finding that seeds stored inside cones may have extended viability, offers important insights into *J. drupacea* seed storage dynamics and seedling emergence in the field.

In terms of final germination (after adjusting for dead and infected seeds), we achieved almost full germination (~95%) after 154 days in optimized light/dark conditions in water agar without pre-chilling. Our findings indicate that *J. drupacea* seeds do not exhibit deep physiological dormancy, contrary to trends observed in other *Juniperus* species. A previous study (Yavuz & Yilmaz 2017) reported that pre-chilling enhances both final germination and germination rate. In our study, although we observe the increase in the rate of germination, we did not observe higher final germination after pre-chilling (for 16 w in the seed lot of 2022). The discrepancies observed between our experimental years can be attributed to methodological refinements rather than biological variation between seed lots (e.g.

changing filter paper to agar). The secondary periods of chilling (2021 seed lot) seemed to have little to no effect to the final germination. The doubling of final germination of the experiment without pre-chilling might suggest a cohort of seeds every year having an absolute necessity for pre-chilling before germination; however the slow and incremental nature of *J. drupacea* seed germination suggest further investigation to prove this speculation. The species seems light insensitive and the final germination both in dark and in alternating lighting condition are similar. Use of GA_3 did not promote germination for the seed lot of 2022 where it was tested. Since germinating seeds at 20 °C and under alternating temperatures resulted in lower final percentages, this may suggest that higher temperatures have a negative effect on seed germination. Compared to other junipers (Tylkowski 2009, 2010), *J. drupacea* does not need a mandatory pre-chilling or warm stratification treatment for the start of germination, suggesting that the seed do not exhibit a form of dormancy. Similarly, the seed quality, in most collection years, is very high with less than 15% of the cones bearing dead seeds. The fully formed seeds exhibiting almost full viability, based on the TTZ tests, in contrast with other junipers, which exhibit high proportions of dead seeds (Tylkowski 2009, 2010; Gruwez et al. 2017).

Under natural conditions, *J. drupacea* cones mature in autumn; the cones either fall from the trees on full maturation or remain on the canopy until a light force is applied. The cones and included seeds, based on our in situ observations, are eaten and dispersed by mammals, e.g. *Sus scrofa* (boars), *Vulpes vulpes* (foxes), *Meles meles* (badgers), and potential rodents, which either consume the entire cones and disperse them within their scats or they open the woody part removing the seeds; dispersal can also happen by woodpeckers in the area (Fig. 5). Seedlings have also been seen to appear directly from open cones buried in the ground (less than 5 cm below ground) which might indicate the opening of the woody cone part by soil fungi, birds etc., with no experimental evidence available, at the moment, for this assumption. After seed dispersal, the temperature remains below 20 °C, usually until May of the next year, when the majority of the new seedlings are also observed in the field. Based on our field observations, we could assume that seeds germinate during the time window between November (immediately after dispersal) and until May, where the temperature is close to the optimal, used in the lab (~ 15–20 °C), and available moisture for their imbibition. The seeds even though germinate extremely slowly, usually taking more than 2 months, they imbibe water as seen by the increase in the size of the seeds in lab experiments, during the first 1–2 weeks after the preparation of the petri dishes. Further investigation is needed to fully understand the nature of this slow germination process, considering no dormancy breaking cues are necessary for the initiation of *J. drupacea* seed germination.

Limited natural regeneration, expressed as low seedling densities (seedlings $ha^{-}1$), has been reported for high-altitude junipers (e.g., *Juniperus excelsa* M.Bieb.) in the Eastern Mediterranean (Douaihy et al. 2013,; Milios et al. 2011, 2013; Stampoulidis et al. 2013), in agreement (Talhouk et al. 2001; Douaihy et al. 2017) with low *J. drupacea* seedling densities, observed throughout this study. Regeneration and recruitment success in junipers may be constrained by embryo anomalies (Gruwez et al. 2013), seed abortion, germination failure, and seedling mortality (García, 2001). Sexual spatial segregation along habitat quality gradients has been linked to higher reproductive effort in female individuals (Ortiz et al. 2002). Furthermore, female junipers (e.g., *J. communis* L. subsp. *communis*) exhibited lower growth rates compared to males, which may be attributed to shifts in sexual maturity and the costs of cone production (Iszkuło & Boratyński 2011), as well as to poorer habitat conditions (e.g., in *J. thurifera* L.) or adverse climatic factors (Iszkuło et al. 2011). In our previous work (Daskalakou et al. 2022a, b), more female individuals were

Fig. 5 Cone and seed *Juniperus drupacea* dispersal: Scats of fox, *Vulpes vulpes*, containing parts of cones (**A**), cones opened by a rodent collector with seeds removed (**B**), cones, without their fleshy outer cover, placed inside the bark of *Juniperus drupacea* individuals by woodpeckers (**C-D**), scats of badger, *Meles meles* (**E**), and boar, *Sus scrofa*, including cones (**F**)

observed in the southern part of Mt. Parnon, with males being dominant in the northern one. In this study, the newly emerged seedlings exhibited high mortality rates, consistent with patterns in long-lived tree species (Fenner 1987; Daskalakou et al. 2019). The low genetic variation exhibited in *J. drupacea* populations on Mt. Parnon (Avramidou et al. 2023), along with the slow regeneration of the species, might hinder the long-term survival of the species' populations in Greece, in contrast with other range restricted relict woody species (Baali-Cherif & Besnard 2005; Besnard et al. 2012). Although recent attempts of the species' explant regeneration were of limited success (Ioannidis et al. 2023), such practices are valuable for conifer propagation for conservation purposes (e.g. Khamushi et al. 2019; Lábusová et al. 2020).

With the mean temperature and the drought period increasing across the mountainous of Greece (IPCC 2023; Daskalakou et al. 2022a), we expect to see higher mortality both in young seedlings and mature individuals of the species. The present study highlights the necessity for conservation actions on *J. drupacea* populations; seeds can be successfully stored in seed banks for long periods and the germination results create the baseline for successfully propagating the species ex situ for conservation purposes (botanical gardens, re-introduction).

Conclusion

The ecophysiology of *Juniperus drupacea* seed germination was examined under varying temperatures and pre-chilling durations, with and without phytohormone treatments; natural regeneration was monitored across the species' distribution on Mt. Parnon (Greece). The seeds do not require pre-chilling to germinate, although 16 weeks chilling significantly increases the germination rate. The seeds are orthodox and can be stored long-term in conventional seed bank conditions, with storage inside the cones increasing the seed longevity. Under natural conditions in the field, *J. drupacea* shows limited regeneration potential, with only few saplings present in monitoring plots. Ex situ collections will provide valuable resources for propagation and plantlet development, supporting subsequent in situ population enhancement based on the lower mortality of saplings (> 1–3 years old). The increased drought observed over the last decade appears to affect *J. drupacea* populations in Greece, with the high mortality of young seedlings likely related to insufficient water during establishment and early growth. Further research and long-term monitoring are required to understand the resilience and sustainability of Syrian juniper forests under climate change.

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Author contribution ED, SO and CAT designed the experiments and methodology for the work, ED and SO conducted the field work and lab experiments, ED and SO analyzed the results, ED and SO wrote the initial manuscript, ED, SO and CAT critically reviewed the manuscript before submission to the journal, ED acquired the funding for the current research.

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Data availability All the data used in the present study are available in the text, the raw data sets produced during the work are available upon request from the authors.

Declarations

Ethics, consent to participate, and consent to publish declarations All authors have been informed in the form of the submitted manuscript, and agreed in its publication.

Competing interests The authors declare no competing interests.

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