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Altitudinal effect on Aleppo Pine (*Pinus halepensis* Mill) Seed Production and Seed Germination in Northeastern Libya

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ABSTRACT

Al Jabal Al Akhdar mountain is located in the south of the Mediterranean Sea in northeastern Libya. It is the only original home of Aleppo pine trees in eastern North Africa and between the Mediterranean Sea and the Sahara Desert. Currently, climate change and human activities are considered the most important factors threatening the existence of this type of tree in this region. In the current study, three *Pinus halepensis* Mill forests at different altitudes above sea level were studied in terms of their seasonal seed productivity, the ability of these seeds to germinate, as well as the ability of seedlings to grow and settle in this unusual and rare ecosystem. The study found that Aleppo pine produces more cones per tree and more seeds per cone than trees in most northern populations, with a net result at higher elevation of more than 7 times the number of seeds per tree compared to the northern Mediterranean. Moreover, seeds from forests at the highest elevation showed better germination, longer root length, higher root mass, higher dry mass and better growth than the other two groups, so they are likely to grow better during intermittent rainfall and be large enough to survive in subsequent dry periods. It is concluded from this study that *P. halepensis* is able to sustain itself in this southern outpost in the short term, but is likely to decline rapidly in the face of climate change and lack of protection. Conservation action are urgently needed to protect and facilitate spread of natural stands.

تأثير الارتفاع عن مستوى سطح البحر على إنتاجية البذور ونمو البادرات في أشجار الصنوبر الحلبي في شمال شرق ليبيا

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الملخص

يقع الجبل الأخضر جنوب البحر الأبيض المتوسط في شمال شرق ليبيا وهو الموطن الأصلي الوحيد لأشجار الصنوبر الحلبي في شرق الشمال الإفريقي وما بين البحر المتوسط والصحراء الكبرى. حالياً، تعتبر عوامل تغير المناخ والأنشطة البشرية من أهم العوامل التي تهدد وجود هذا النوع من الاشجار في هذه المنطقة. في الدراسة الحالية، تم دراسة ثلاث غابات من *Pinus halepensis* Mill على ارتفاعات مختلفة من سطح البحر، من حيث إنتاجيتها الموسمية للبذور ومقدرة هذه البذور على الإنبات وكذلك مقدرة البادرات على النمو والاستقرار في هذا النظام البيئي غير العادي والنادر. وجدت الدراسة أن الصنوبر الحلبي في منطقة الدراسة ينتج المزيد من المخاريط لكل شجرة والمزيد من البذور لكل مخروط أكثر من معظم الأشجار التي تنمو في المناطق التي تقع شمال المتوسط، مع اجمالي يزيد عن سبعة أضعاف عدد البذور لكل شجرة مقارنة

الكلمات الدالة:
الصنوبر الحلبي
علم البيئة الذاتية
إنبات البذور
صنوبر هالبينسيس
إنتاج المخاريط والبذور
نمو البادرات

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لدراسات أخرى لمناطق تقع شمال البحر الأبيض المتوسط. علاوة على ذلك، أظهرت البذور من الغابات على ارتفاعات أعلى عن مستوى سطح البحر في منطقة الدراسة إنباتا أفضل، وطول جذر أطول، وكتلة جذر أعلى وكتلة جافة أعلى ونموا أفضل من المجموعتين الأخريين، لذلك من المرجح أن تنمو بشكل أفضل أثناء هطول الأمطار المتقطع وتكون كبيرة بما يكفي للبقاء على قيد الحياة في فترات الجفاف اللاحقة. نستنتج من هذه الدراسة أن *P. halepensis* قادر على الحفاظ على نفسه في هذه البؤرة الاستيطانية جنوب المتوسط على المدى القصير، ولكن من المرجح أن يتدهور بسرعة في مواجهة تغير المناخ ونقص الحماية. هناك حاجة ماسة إلى إجراءات عملية لحماية الغابات والأنواع النباتية في هذه المنطقة والعمل على زيادة رقعة انتشار الغابات الطبيعية.

1. Introduction

Pinus halepensis Mill. (Aleppo pine: Pinaceae) is a native tree species to the Mediterranean basin and distributed naturally from eastern Portugal in the west to Palestine, Jordan, Lebanon and South West Syria in the east, and from northern France to northeast of Libya in the south [1]. It naturally covers an area of ~3.5 million hectares, mostly in northern Mediterranean, but in the south, it is restricted to areas in Morocco, Algeria, Tunisia (~1.3 million hectares) and Libya [2]. Despite its small native range, it is a versatile tree able to grow at a range of altitudes and cope with low precipitation. Indeed, *P. halepensis* has been introduced to huge areas of the USA, Australia and South Africa from the 19th century [3].

Aleppo pine is considered as one of the tree species in the Mediterranean with the largest ecological amplitude and can survive and succeed in semiarid areas [4]. Aleppo pine forests grow naturally at elevations from sea level and up to 2600 m [4] and in habitats characterized by warm and cold winters, and dry summers with extended periods of drought with optimal annual precipitation ranges from 350 to 750 mm [2]. As a phytosociologically dominant species, *P. halepensis* is the defining species for Eunis habitat type G.3.74 and for several alliances in the classification of Mediterranean pine forest vegetation proposed by [5]. Its timber is locally important, particularly for charcoal production, and the pine seeds are used in cooking. Perhaps more significantly it provides a number of ecosystem services including improving water infiltration, preventing soil erosion on dry slopes and serving as a windbreak [6], [7]. Aleppo pine has played an important role in protecting soils, combating desertification, supplying natural resources and providing space for recreation [8], [9], [10], [11].

However, in the eastern half of North Africa, Aleppo pine can be naturally found only in the Al Jabal Al Akhdar area in the northeast of Libya and associated with *Juniperus phoenicea* L. and *Cupressus sempervirens* L. These communities form the majority of natural forest land in this area covering more than 217 000 ha, less than 0.1% of the total area of Libya [12]. Within Al Jabal Al Akhdar, *P. halepensis* is associated with a diverse understory flora, including a number of endemics, and a rich woodland fauna [13], [14], [15], [16]. From the mid-1970s and for more than a decade, the authorities in Libya made a great effort to limit desertification and to reestablish native tree species such as Aleppo pine in extensive areas along the coastline, although no quantitative data are available.

Aleppo pine is entirely regenerated by seeds, and there are several factors that contribute to its diversity of annual seed production. These factors include: (i) individual tree size [17], [18], [19], [20], (ii) stand characteristics [21], [22], (iii) tree density [23], (iv) geographic variables [24], [25], (v) years with or without abundant fructification [18], (vi) environmental factors [19] and (vii) genetic effects [26]. Many studies concluded that larger trunk diameter (DBH) of Aleppo pine trees are associated with bigger cones and higher number of seeds [20]. Also, in some areas around the Mediterranean, tree height and crown diameter also affected cone and seed production in Aleppo pine [18], [20], [27], [28]. Reproduction in Aleppo pine is a long process that takes more than 3 years. Therefore, the growth and development of seeds and cones may be exposed to harsh climatic conditions in the dry areas of Libya. Seed shedding can be delayed due to partial serotiny which is the long-term retention of seeds in a plant canopy, further exposing the seeds to stressful conditions [29].

The effect of altitude on Aleppo pine seed production is not entirely clear, as several studies in North Africa have concluded that increasing altitude improves the production of Aleppo pine cones and seeds, and the continental gradient may have a positive effect on these characteristics in this region [24], [25]. Also, the average individual seed mass and cone size increased with increasing altitude [4], [24]. But there was an interaction between the effect of latitude and longitude in this region, since Aleppo pine trees in the east produces fewer cones and smaller seeds [24], [25]. Furthermore, another study concluded that cone size was less affected by altitude than seed mass; and longitude and latitude had more effect on seed and cone production in this species than elevation [19]. In contrast, in northern Mediterranean, several studies showed negative effects of altitude on Aleppo pine cone and seed production, as cone abortion can occur in cold regions and impede seed maturation [22], [30].

The long-term future of Aleppo pine at the southern edge of its natural range depends upon continued reproduction. However, it is clear from the morphology and extensive shoot dieback that Aleppo pine populations in this region are suffering from environmental stress. Yet, it is unclear what factors control seed production and successful seedling establishment. Therefore, the aim of this study was to determine how the current capability of Aleppo pine in this region to produce cones and seeds was affected by tree size and altitude, and the ability of these seeds to germinate and establish successfully linked to water availability. This can be used to determine the long-term future of Aleppo pine in this region, and the longitudinal and latitudinal effect on cone and seed mass and production in the entire of North Africa.

2. Materials and Methods

2.1. Area of Study

Three sites in northern Libya (Fig. 1) were chosen based on the extensive area of *Pinus halepensis* woodlands. Site A was at Albacore 32°30'56.7"N 20°37'39.4"E in the Marj district located 75 km east of Benghazi city and at 181m above sea level. Site B was at Wadi Alkouf 32°40'29.2"N 21°32'59.6"E located in the Al Jabal Al Akhdar district at 327 m a.s.l. and the last, site C was at Sidi Alhomry 32°38'15.6"N 21°47'58.3"E located in the Al Faidia district at 843 m a.s.l. Records suggest that site B is a remnant of natural vegetation whereas the other sites were plantations established by the agriculture ministry in the 1980s using local seeds sourced in the Al Jabal Al Akhdar area.

The entire area is characterized by a Mediterranean climate, with a hot summer and slightly wet and cold winter, while the annual precipitation does not exceed 400 mm. The three sites were similar climatically, with the highest daily average temperature between June and September with 30 °C in July and August in both sites B and C, sites B and C showed higher temperature than site A which is located close to the shoreline. Precipitation was mostly between October and February; annual precipitation was lowest at site A (297 mm per annum) and similar at sites B and C (354 and 356 mm, respectively) (Table 1) [31].

2.2. Tree Density

Ten quadrats, each 10 x 10 m, were randomly located at each site;

the number of all tree individuals was counted in each quadrat. Trunk diameter at breast height for each tree was also measured. The total area of each site was determined by Google Earth software. The frequency and density of trees were calculated. In all sites, Aleppo pine was dominant and recorded in all quadrats.

2.3. Cone Collection

Aleppo pine trees were randomly selected from each site (Fig. 1) The intensive sampling within each tree necessitated that this was limited to six trees per site. All cones on each tree were visually counted by dividing the whole crown into four parts, with the assistance of the leader and coloured rope and climbing the tree when needed. One hundred cones were randomly collected throughout the canopy from each tree in July 2018. Tree height, and trunk diameter were measured. Age of trees was determined by ring counts taken at the base using an increment corer, 6 mm in diameter [32].

2.4. Dealing with Cones

Cones were air-dried in the sun for one week at temperatures between 30 and 35°C. Fifty cones were randomly sampled from each tree. Length, width and mass of sampled cones were measured. Sampled cones were placed individually in ventilated plastic cups and left in the sun for two more weeks to open the cones enough to release their seeds. Seeds were manually shaken from the cones, counted, and as a tree, weighed. Mean seed mass was determined for each tree using ten replicate batches of 100 seeds that were randomly sampled [32].

2.5. Germination Experiment

Seeds sampled were sterilized in 5% sodium hypochlorite solution for 5 minutes. Mean germination of each seed source was assessed by selecting 10 seeds randomly from each tree with 3 replicates and germinating them in 9 cm diameter Petri dishes on a double layer of Grade 2 Whatman filter paper. Mean germination of each site was calculated [33].

The effect of water regime on germination and early growth was tested by sampling 10 seeds from each of 6 trees from each site. These were sown in 9 cm Petri dishes on filter paper (Whatman # 40). Three replicate plates were treated separately with 9 different watering regimes of 1, 3 and 6 ml every 3, 6 and 9 days. The lowest amount of water was enough to wet the paper without forming an annulus around each seed. Preliminary studies showed that these amounts allowed differentiation between germination behaviour at different sites. The viability of non-germinating seeds was not tested [34].

The Petri dishes were kept at room temperature (21±2ºC) and 14 hours daily light for three weeks. Seedlings were monitored daily. Shoot and root lengths, and dry mass of the young seedlings were measured at the end of the experiment to give an indication of their future vigour [34].

2.6. Statistical Analysis

One-way ANOVA was used to assess seed production per tree and tree differences across tree height, age and trunk diameter. The same test was used to compare cone parameters, seed mass and viability. For water regime data, one and two way ANOVA were used to investigate the differences between seed germination and seedling performance with site elevation and different watering regime, followed by Tukey's or Fisher's post hoc paired comparisons between means. Data are presented as mean ± standard deviation. Minitab® Version16 software was used for analyses.

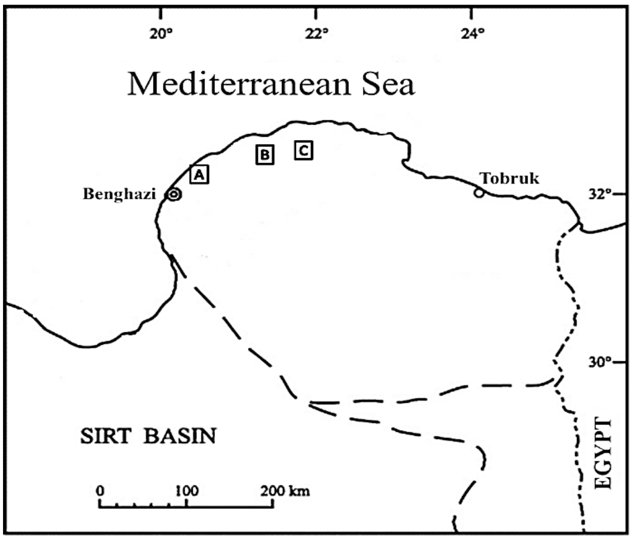

Figure 1. Location of the three sites studied in Al Jabal Al Akhdar in Cyrenaica in north-east Libya. Site A = Albacore (181m a.s.l.), Site B = Wadi Alkouf (327m a.s.l.), Site C = Sidi Alhomry (843m a.s.l.).

Table 1. Average monthly maximum and minimum temperature (°C) and average monthly precipitation (mm) in the region of the three sites in the period between 2016 and 2019.

Month	Daily Max. Temperature °C			Daily Min. Temperature °C			Average Monthly Precipitation (mm)		
	Site A	Site B	Site C	Site A	Site B	Site C	Site A	Site B	Site C
January	15	15	15	9	9	9	34	45	45
February	15	16	16	9	10	7	57	67	67
March	17	18	18	10	11	8	15	18	18
April	19	22	22	11	13	10	10	14	14
May	22	25	25	13	16	12	8	12	12
June	25	29	28	15	20	14	2	1	3
July	26	30	30	16	22	16	2	0	0
August	28	30	30	19	22	17	1	2	2
September	26	28	28	17	21	15	22	21	21
October	24	25	25	15	18	13	43	59	59
November	21	21	21	13	15	11	43	41	41
December	17	16	16	11	11	9	60	74	74
Annual Mean	21.2	23.1	23.0	13.3	15.5	11.5	298	354	356

***Source**: Climate Data (2023) for northern Libya [31].

3. Results

3.1. Trees and Cones

The mean height of trees in all sites was 9.1±0.71 m (Table 2). The tallest individual tree (16 m; see Supplementary material) was found at site C which is located on the highest elevation above the sea level, and the shortest tree (4 m) was recorded in site A at the lowest elevation. Trees at site C (12.3±1.12) were significantly taller compared to the two other lowest sites (7.3±1.05 and 8.7±0.84 m, respectively) (Table 2).

Aleppo pine tree trunk diameter ranged from 10.2 cm in site B to 26 cm in site C (Appendix 1) but no significant differences of elevation on tree trunk diameter were found (Table 2).

Age of Aleppo pine trees sampled over all sites ranged from 13 y in site B to 44 y in site C (Appendix 1) with an overall mean of 29.8±2.24 y (Table 2). There was a significant effect between sites in tree age as site B had the youngest trees 18.8±1.54 y which was significantly different from sites A and C which were 32.5±1.29 and 38.2±2.73 y, respectively (Table 2).

The productivity of cones of individual trees was very variable and ranged between 52 cones in site B and 1093 cones in site C (Appendix 1). Trees on site C produced more cones and was significantly different from the two other sites. Mean number of cones per tree in sites A to C were 114±32.1, 230±139.4 and 1018±55.5 cones, respectively. The mean number of cones produced per tree over all the area of study was 454±108.1 cones (Table 2).

Cone shape varied between sites. The longest individual cones were found in site C (10.1±1.03 cm), whereas the shortest cones were recorded in site A (5.8±0.66 cm) (Appendix 1). Cones from all sites were significantly different with the longest mean cone length at site B (9.1±0.33) (Table 2). However, mean cone width was significantly greater at site C (4.6±0.26 cm) and shortest at site A (2.6±0.19 cm) (Appendix 1). The mean cone width over all sites was 3.4±0.14 cm (Table 2).

3.2. Seed Production

The highest mean number of seeds per cone was found in a tree in site C with 103.8±14.8 seeds, as was the lowest with 44.9±16.73 seeds (Appendix 1). The overall mean number of seeds per cone was lowest at site A (61.7±3.57 seeds) and was significantly higher at sites B and C at 76.0±7.31 and 77.1±8.95 seeds, respectively. The mean number of seeds per cone over the whole study area was 71.6±4.15 seeds (Table 2).

The highest mass of 100 seeds was recorded in a tree in site C with 3.6±0.11 g, whereas the lowest was in site A with 1.1±0.12 g (Appendix 1). The overall mean mass of 100 seeds was significantly different between sites with the heaviest seeds (2.7±0.23g) at site C. The overall mean mass of 100 seeds in this project was 2.0±0.17 g (Table 2).

Pine density was significantly different between sites; site C contained the most at 15.7±2.79 trees per 100 m^2 which was significantly higher than sites A and B which had means of 4.6±0.65 and 4.3±0.93, respectively. Across all sites, the density of Aleppo pine was 8.2±1.38 tree per 100 m2 or 820±13.8 tree per hectare (Table 3). Estimated population size ranged from 1166±252.2 trees (431±93 tree/ha) at site B to 81064±3665.7 trees (1571±71 tree/h) at site C (Table 3). Based on this and mean seed production per tree, the seed productivity in 2018 was estimated at almost 14 million at site A, almost 21 million at site B and >6 billion at site C (Table 3).

3.3. Seed Germination and Seedling Performance

The overall germination of Aleppo pine seeds under optimum conditions was 49.8±3.19%. Seeds from site C, which is the highest altitude site, showed significantly higher germination (65.0±4.52 %) than seeds from sites A and B (37.2±4.10 and 47.2±5.10, respectively) (Fig. 3).

Generally, one-way ANOVA showed significant differences in seed germination among trees within sites A and B (F (5, 17) =11.93; $P<0.001$ and F (5, 17) =19.65; $P<0.001$, respectively). But no significant differences were found among trees within site C (Appendix 1). With the same case scenario, one way ANOVA showed within sites A and B significantly higher germination of seeds from taller trees, those with bigger trunk diameter and from older trees (Appendix 1). But, no significant differences among trees were shown within site C (Appendix 1).

Under the different water regimes used in this trial, seeds from site C showed an overall germination of 34.8±28.77 % which was significantly higher than in seeds from sites A and B which were 13.89±18.20 % and 19.75±22.95 %, respectively (Fig. 4).

Under different water regimes and irrespective of the site, one-way ANOVA showed significant differences between water regimes (F(8, 161) = 16.60; ($P<0.001$), with the highest mean germination percentage using a water regime of 3 ml per 6 days (46.9±23.67%) which was significantly different from water regimes of 1ml/6d, 1ml/9d, 6ml/3d and 6ml/6d (Fig. 4).

Table 2. Parameters of sampled Aleppo pine trees and cones. Different letters in each column indicate significance. F values for statistical analysis between sites are given; ns non-significant, +++ $p<0.001$.

Site	Elevation a.s.l. (m)*	Tree height (m) (*n*=6)	Trunk diameter (cm) (*n*=6)	Age (y) (*n*=6)	Number of cones/tree (*n*=6)	Cone length (cm) (*n*=300)	Cone width (cm) (n=300)	Mean Number of seeds/cone (*n*=300)	Mean of 100 seed mass (g) (*n*=60)
A	181	$7.33±1.054^b$	$20.9±2.20^a$	$32.5±1.29^a$	$114±32.1^b$	$7.1±0.64^c$	$2.9±0.14^c$	$61.7±3.57^b$	$1.3±0.05^c$
B	327	$8.67±0.843^b$	$15.8±2.28^a$	$18.8±1.54^b$	$230±139.4^b$	$9.1±0.33^a$	$3.4±0.19^b$	$76.0±7.31^a$	$2.1±0.15^b$
C	843	$12.25±1.124^a$	$22.1±1.67^a$	$38.2±2.73^a$	$1018±55.5^a$	$8.4±0.40^b$	$3.9±0.17^a$	$77.1±8.95^a$	$2.7±0.23^a$
All sites		9.06±0.712	19.6±1.30	29.8±2.24	454±108.1	8.2±0.33	3.4±0.14	71.6±4.15	2.0±0.17
		$F_{(2, 17)}=9.39^{+++}$	n.s.	$F_{(2, 17)}=25.91^{+++}$	($F_{(2, 17)}=30.9^{+++}$	($F_{(2, 899)}=78.1^{+++}$	($F_{(2, 899)}=59.74^{+++}$	($F_{(2, 899)}=35.51^{+++}$	($F_{(2, 180)}=205.60^{+++}$

*Elevation above the sea level.

Table 3. Area of each site and density of individuals, estimated from ten 10 x 10 m^2 quadrats at each site. *F* values for statistical analysis between sites are given; +++ $p<0.001$.

Site	Area (m^2)	Mean Number of individuals/100m^2	Estimated number of individuals/sites*	Estimated number of cones/sites**	Estimated number of seeds/trees***	Estimated number of seeds/sites****	Estimated seed mass produced/site (kg)*****
A	43 214	$4.6±0.65^b$	1988±280.9	226632	6981.1±1962.71	13938593±2591708.3	180.4
B	27 116	$4.3±0.93^b$	1166±252.2	268180	17972.0±10858.88	20955352±7922218.7	440.1
C	516 331	$15.7±0.71^a$	81064±3665.7	82523152	78742.48±9883.09	6383141488±492086031.9	172 344.8
All Sites	586 661	8.2±0.81	84218±1399.6	38234972	34565.1±8949.82	-	-
-	-	$F_{(2, 29)}=13.99^{+++}$	-	-	-	-	-

* Estimated number of individuals per site was calculated based on the mean number of individuals/100m^2 and the total area of the site.

** Estimated number of cones per site was calculated by multiplying estimated number of individuals per site and mean number of cones per tree on the site.

*** Estimated number of seeds per tree was calculated as total estimated number of seeds per cone multiplied by the mean number of cones per tree

**** Estimated number of seeds per site was calculated as total estimated number of trees multiplied by the estimated number of seeds per tree.

***** Estimated seed mass produced/site (kg) was calculated by multiplying the total estimated number of tree individuals in the site with the estimated number of seeds per tree, divided by the mean mass of 100 seeds in the site.

Within site A, there were significant differences in germination percentage between water regimes. Watering of 1ml/3d, 3ml/6d and 3ml/9d produced the highest germination percentage of 25.0±19.41, 28.3±17.09 and 37.2±21.02 %, respectively. Lowest germination was in treatments of 1ml/6d, 1ml/9d, 3ml/3d, 6ml/3d and 6ml/6d with 0, 0, 10.0±6.67, 0 and 8.9±11.67, respectively (Fig. 4). In addition, within site B, there were significant differences in germination percentage between water regimes. Watering of 1ml/1d, 3ml/6d and 6ml/9d had the highest value of 37.2±22.45, 47.2±21.75 and 40.6±20.48, respectively and the lowest were treatments of 1ml/6d, 1ml/9d, 3ml/3d, 6ml/3d and 6ml/6d with 0, 0, 14.4±6.89, 0.6±1.36 and 6.1±9.24, respectively (Fig. 4). Furthermore, within site C, there were significant differences in germination percentage between water regimes. Watering of 1ml/1d, 3ml/3d, 3ml/6d and 6ml/9d were the highest with 64.4±21.95, 47.8±18.22, 65.0±18.23 and 55.0±23.73, respectively (±SD) and the lowest were treatments of 1ml/6d, 1ml/9d, 3ml/9d, 6ml/3d and 6ml/6d with 1.7±4.08, 0, 31.7±26.89, 16.7±10.54 and 31.7±15.02, respectively (Fig. 4).

Two-Way ANOVA showed significant interactions between site and water regime in germination percentage (F (16, 161) =2.55; $P<0.01$). There is sufficient evidence to conclude that the type of water regime is related to percent germination. However, the significant interaction indicates that relationship between water regime and germination depends on the site. Consequently, water regime of 1ml/day, 3ml/3days, 6ml/6days and 6ml/9days showed high value of germination percentages which were increased at higher attitude.

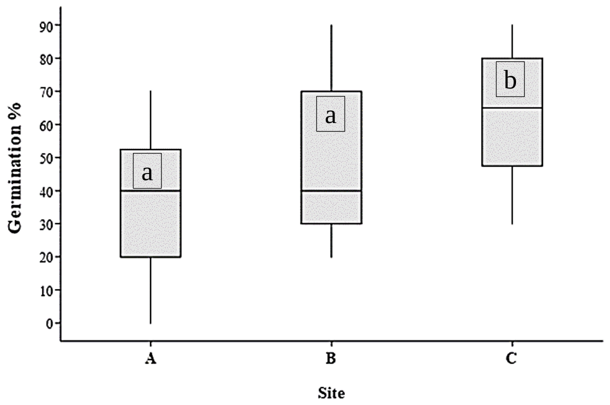

Figure 3. Germination percentage of Aleppo pine seeds from the three sites A, B and C. (n=18).

Different letters indicated significant differences ($F_{(2, 53)}$ =8.20, P<0.01). In the box plots, the boundary of the box closest to zero indicates the 25th percentile, a black line within the box marks the median and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 10th and 90th percentiles. The longest shoot of an individual over the three weeks of measurement was 80 mm in the treatment of 6ml/3day at site B (n=1).

The overall mean shoot length was highest at 56.8±23.10 mm in treatment 6ml/9days at site A (n=28), whereas the shortest mean shoot length was 30.18±15.44 mm in treatment 3ml/9days site B (n=11) (Fig. 5). No significant differences were found between sites in shoot length.

One-way ANOVA showed highly significant differences between different water regime across the experiment and regardless of site. The longest length of seedlings were showed in treatment of 6ml/9days and 3ml/6days with means of 50.21±23.63 (n=199) and 48.37±22.78 mm (n=253), respectively, which were significantly different from the rest of water regimes (Fig. 5).

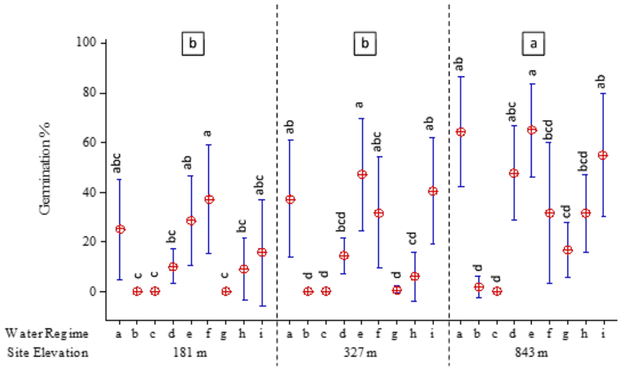

Figure 4. Germination percentage of Aleppo pine seeds from each site under different water regimes. Site A = Albakour, 181 m a.s.l.; site B = Wadi Alkouf, 327 m a.s.l.; site C = Sidi Alhomry, 843 m a.s.l. Water Regime: a = 1ml/3days, b = 1ml/6days, c = 1ml/9days, d = 3ml/3days, e = 3ml/6days, f = 3ml/9days, g = 6ml/3days, h = 6ml/6days, i = 6ml/9days.

Different letters indicated significant differences ($F_{(2, 161)}$ =11.27; P<0.001) between sites; ($F_{(8, 53)}$ =5.91; P<0.001) within site A, ($F_{(8, 53)}$ =10.20; P<0.001) within site B, ($F_{(8, 53)}$ =12.35; P<0.001) and within site C. n=6, ±SD.

Within site A, there were significant differences in shoot length, with the highest in treatment 6ml/9d and 3ml/6d with 56.77±23.10 (n=28) and 48.6±20.16 mm (n=51), respectively, and the shortest shoots were in treatments of 3ml/9d, 6ml/6d and 1ml/3d with 35.77±15.67 (n=66), 36.9±22.20 (n=16) and 40.6±13.46 mm (n=47), respectively (Fig. 5). In site B, one way ANOVA showed significant differences between treatments. Irrespective of treatment 6ml/3d, which had only one seedling, the highest mean of shoot length was in 6ml/9d and 3ml/6d at 54.0±23.26 (n=73) and 47.8±22.34 mm (n=85), respectively. The shortest shoots in this site were 3ml/9d, 6ml/6d, 3ml/3d and 1ml/3d at 30.2±15.44 (n=57), 30.9±23.96 (n=11), 34.4±22.01 (26) and 36.2±14.75 mm (n=67), respectively (Fig. 5). Also, within site C, there were significant differences between treatments, the longest were 3ml/6d and 6ml/9d at 48.7±24.31 (n=31) and 45.5±23.33 mm (n=98), respectively. These were significantly different from the shortest treatments of 1ml/6d and 1ml/3d with 15.0±5.29 (n=3) and 35.4±18.16 mm (n=116), respectively (Fig. 5).

One way ANOVA showed significant differences between the three sites in root length of seedlings. Root length in site C was significantly longer than sites A and B: 35.3±21.21 (n=565), 30.0±14.34 (n=226) and 31.4±16.34 mm (n=320), respectively (Fig. 6). Irrespective of site, one-way ANOVA showed significant differences between different water regimes, as treatments of 3ml/9day and 1ml/3day produced the longest roots at 42.7±15.63 (n=180) and 40.0±18.37 (n=230), respectively. By comparison, the 6ml/6day treatment produced the shortest roots at 17.3±15.47 (n=84) (Fig. 6).

In site A, 3ml/9d root length (39.6±13.15, n=66), was significantly longer than in other treatments. Treatments of 6ml/6d, 3ml/3d and 6ml/9days produced the shortest roots at 16.3±9.57 (n=16), 21.4±11.98 (n=18) and 26.7±15.45 mm (n=28), respectively.

Again, in site B, the treatment of 3ml/9d showed the longest roots (40.79±15.35, n=57) compared to other treatments. The treatment of 6ml/3d was longer but was represented by only one seedling.

Shortest roots in this site were showed in treatments of 6ml/6d and 3ml/3days at 11.8±4.05 (n=11) and 19.8±12.37 mm (n=26), respectively. In site C, treatments of 1ml/3d and 3ml/9d showed the longest roots at 84.7±19.07 (n=116) and 48.3±17.25 mm (n=57), respectively, and both were significantly different from other treatments. Treatments of 6ml/6d, 6ml/3d, 3ml/3d and 1ml/6days produced the shortest roots: 18.7±17.87 (n=57), 23.7±13.13 (n=31), 27.2±20.39 (n=86) and 31.0±1.73 mm (n=3), respectively (Fig. 6).

One way ANOVA showed significant differences between the three sites in shoot dry mass of seedlings; site C was significantly the highest followed by site B and A: 8.96±4.109 (n=562), 6.86±2.708 (n=226) and 5.47±1.993 mg (n=320), respectively (Fig. 7).

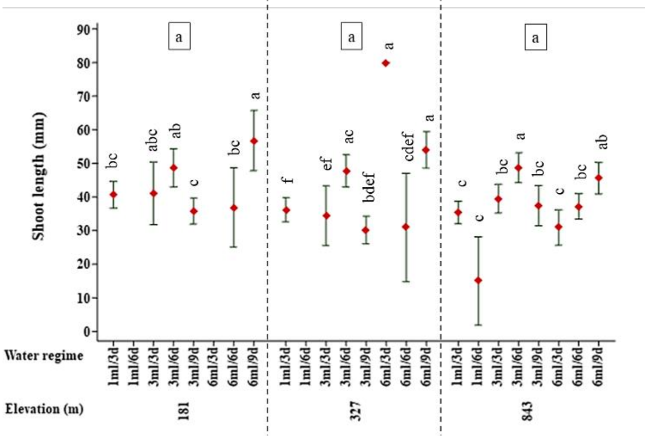

Figure 5. Mean (±SD) shoot length of seedlings of Aleppo pine from each site under different water regimes.

Watering regime of 1 ml per 9 days produced no seedlings at any site; 1 ml per 6 days and 6ml per 3 days were missing in the first site and 1 ml per 6 days was missing in site B for the same reason. Different letters indicated significant differences ($F_{(7, 1110)}$ =17.32; P<0.001) between different water regime overall the experiment and regardless of site; ($F_{(5, 225)}$ =6.85; P<0.001) within site A, ($F_{(6, 319)}$ =11.47; P<0.001) within site B, ($F_{(7, 564)}$ =6.51; P<0.001) and within site C, and no significant differences between sites.

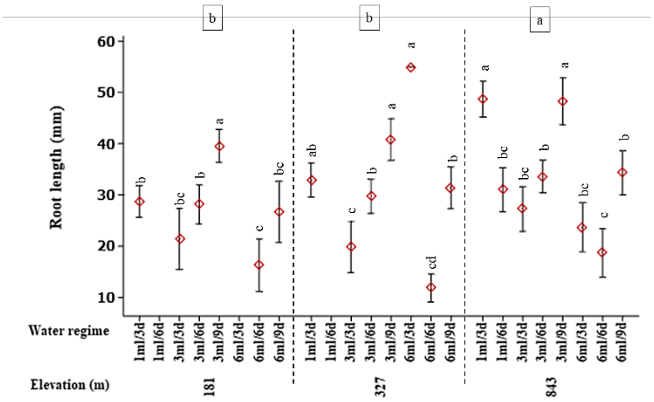

Figure 6. Mean (±SD) root length of seedlings of Aleppo pine from each site (See Fig. 4) under different water regimes.

Watering regime of 1 ml per 9 days produced no seedlings at any site; 1 ml per 6 days and 6ml per 3 days at site A and 1 ml per 6 days at site B are missing for the same reason. Different letters indicated significant differences (F (2, 1110) =8.20; *P*<0.001) between sites; (F (5, 225) =13.49; *P*<0.001) within site A, (F (6, 319) =9.99; *P*<0.001) within site B, (F (7, 564) =22.78; *P*<0.001) and within site C, and (F (7, 1110) =28.90; *P*<0.001) between different water regime overall the experiment and regardless of site.

There were however differences within sites. Within site A, there were significant differences were found in water regime by Fisher's pair ways teat. The highest shoot dry mass was in treatments of 6ml/9d and 3ml/9d: 6.04±1.991 (n=28) and 5.86±1.936 mg (n=66), respectively.

The smallest shoot mass was in 6ml/6d and 3ml/3d: 4.39±2.019 (n=16) and 4.63±1.998 mg (n=18), respectively. For site B, one way ANOVA showed high significant differences in water regime, the highest mass were in treatments of 6ml/3d (but was represented with only one seedling), 6ml/9d, 3ml/6d, 1ml/3d and 3ml/9d: 7.21±2.770 (n=73), 7.17±2.772 (n=85), 7.14±2.256 (n=67) and 6.92±2.617 mg (n=57), respectively. The lowest mass was in 6ml/6d at 3.81±2.507 (n=11). There was no significant difference in water regime within site C (Fig. 7). One way ANOVA showed significant differences between the three sites in root dry mass of seedlings and in site C were significantly higher (2.31±1.404 mg, n=561), followed by site B and A at 1.75±1.397 (n=320) and 1.49±0.891 mg (n=226), respectively (Fig.8).

Leaving aside the site, there were high significant differences within water regimes, since treatment of 1ml/6d showed the highest mean of root dry mass over the experiment at 2.87±0.577 mg (n=3) followed by 1ml/3d at 2.50±1.333 mg (n=229) and the lowest mean of root dry mass was 6ml/6d at 1.07±1.037 mg (n=82) (Fig. 8).

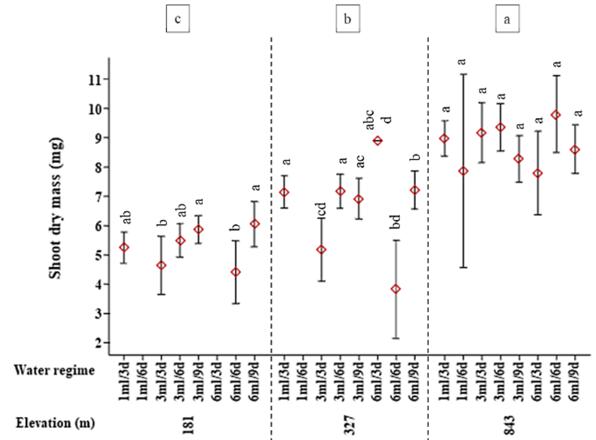

Figure 7. Mean (±SD) shoot dry mass of seedlings of Aleppo pine from each site (see Fig. 4) under different water regimes.

Watering regime of 1 ml per 9 days produced no seedlings at any site; 1 ml per 6 days and 6ml per 3 days at site A, and 1 ml per 6 days and 6 ml per 3 days at site B are missing for the same reason. Different letters indicated significant differences (F (2, 1107) =97.33; *P*<0.001) between sites; (F (5, 225) =2.79; *P*<0.001) within site A, (F (6, 319) =4.97; *P*<0.001) within site B and no significant differences were found within site C.

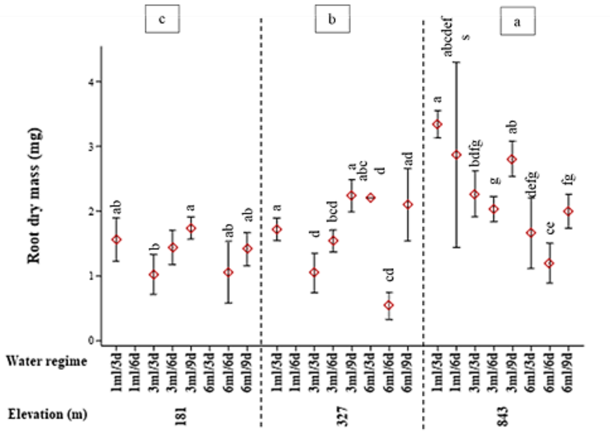

Figure 8. Mean (±SD) root dry mass of seedlings of Aleppo pine from each site (see Fig. 4) under different water regimes.

Watering regime of 1 ml per 9 days produced no seedlings at any site; 1 ml per 6 days and 6ml per 3 days at site A, and 1 ml per 6 days at site B are missing for the same reason. Different letters indicated significant differences (F (2, 1106) =38.32; *P*<0.001) between sites; (F (5, 225) =3.08; *P*<0.05) within site A, (F (6, 319) =5.14; *P*<0.001) within site B, (F (7, 560) =21.80; *P*<0.001) and within site C, and (F (7, 1106) =13.64; *P*<0.001) between different water regime overall the experiment and regardless of site.

Within site A, the 3m/9d treatment had the highest root dry mass at 1.74±0.689 mg (n=66) and was significantly different from 3m/3d, and treatment of 3m/3d was the lowest with 1.02±0.620 mg (n=18) (Fig. 8). Site B also showed significant differences in water regimes, the highest were treatments of 3ml/9d and 6ml/9days at 2.23±0.938 (n=57) and 2.10±2.406 mg (n=73), respectively. The lowest were 6ml/6d, 3ml/3d and 3ml/6days at 0.53±0.313 (n=11), 1.04±0.755 (n=26) and 1.53±0.787 mg (n=85), respectively. Site C had also the same similarity, there were significant differences in root dry mass, the treatment of 1ml/3d was the highest at 3.34±1.134 mg (n=115) and 6ml/6d was the lowest at 1.19±1.138 mg (n=55) (Fig. 8).

Root : shoot ratio was not significantly different between sites (Fig. 9). However, there were no significant differences within sites A and B (Fig. 9). Within site C, the root: shoot ratio was significantly higher in the 1ml/3d treatment (0.435±0.2180, n=115) than in the 6ml/6d treatment (0.163±0.2180, n=53).

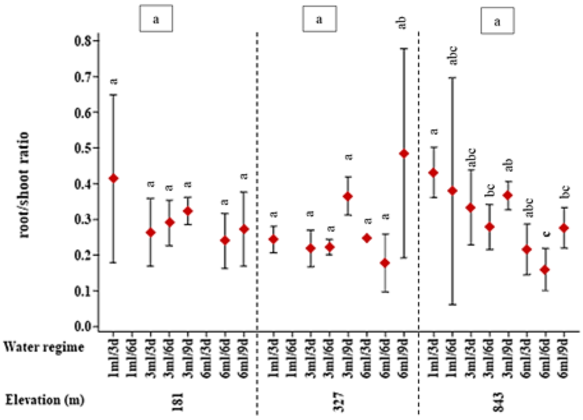

Figure 9. Mean (±SD) root : shoot ratio of seedlings of Aleppo pine from each site (see Fig. 4) under different water regimes.

Watering regime of 1 ml per 9 days produced no seedlings at any site; 1 ml per 6 days and 6ml per 3 days at site A, and 1 ml per 6 days at site B are missing for the same reason. Different letters indicated significant differences. Only significant differences were found within site C (F (7, 556) =4.46; *P*<0.01).

4. Discussion

4.1. Tree Size and Age

Aleppo pine is capable of reaching 20 m in height with a DBH of 80 cm and living for up to 150-200 y [34]. Aleppo pine trees around the Mediterranean begin producing seeds at 4-7 years old with highest seed production between 12 and 100 y [1], [34].

From this study, tree age ranged between 32 and 38 y, with the oldest trees at site C, the highest site above sea level.

The young age and small diameter of the study trees fits with the plantation origin of sites A and C but indicates that even the more natural site B has been extensively managed or has suffered extensive mortality of older trees due to past severe droughts. However, this puts the trees sampled within the most seed productive age of Aleppo pine in the Mediterranean [1].

4.2. Seed Production

Within its natural range, Aleppo pine cones range between 6.6 and 11.6 cm in length and between 6.4 and 9.0 cm in width [23], [35], with typically a maximum of 242 cones per tree [23]. The average number of seeds per cone ranges from 25 in Greece to 79 in Spain [18], [35]. This results in a typical Aleppo pine producing around ten thousand seeds annually [23]. Seed mass varies widely between areas ranging from 1.2 to 3.0 g per 100 seeds [24].

It is clear from this study that the trees in northern Libya are producing cones at the smaller end of the range (Table 2) but are producing more cones per tree (mean 454 ± 108.1 across all sites), especially at higher altitude. These also contain more seeds per cone than normal (mean 71.6 ± 4.15 across all sites). The net result is an estimated mean seed production of more than 34,500 seeds per tree across sites, with the higher altitude site C producing more than 75,000 seeds per tree (Table 2), compared to the norm quoted above of 10,290.

Aleppo pine seeds have no natural dormancy [36] and have been found to have very high germination rates ranging from 72 to 80% in both shaded and unshaded environments [36]. Germination in the current study was lower (mean 49.8 ± 3.19 %) but this is more than compensated for by the high seed production per tree. This is especially true of the highest altitude site C where germination was significantly higher than at lower sites (Figure 3).

The optimum temperature for Aleppo pine seed germination is between 15 and 20°C [36]. In this study, germinating seeds at room temperature (21-25°C) might have reduced germination. The viability of un-germinated seeds was not tested due to the lack of suitable chemicals at the time, so it is possible that these seeds were viable but reluctant to germinate under the experimental conditions. Moreover, the seeds were sterilised before being sown and were watered with distilled water, which may have altered the pH of the petri dishes, potentially reducing germination. Certainly, the remaining ash from burned forests can decrease *P. halepensis* germination due to pH changes [37].

However, light levels would have had no effect since germination has been found to be above 80% under both high light and shade treatment [36].

Most importantly, although overall germination was lower than expected, at the lower sites (A and B) this study found significant links between seed germination and variation in tree age, height and tree trunk diameter. This has not previously been recorded for pine species [38].

Although climate change is highly likely to put these trees under increased stress in the future [39], it is clear that seed production is currently high, ensuring a continuous supply to seeds at least in the short term. The reproductive cycle from embryo to mature seed takes 3 years [40]. Moreover, cones of Aleppo pine can be partially or fully serotinous, releasing seeds following very dry conditions [23], [41]. Both these factors increase the temporal separation of seed production and release, which may make seed production more sensitive to climate change and so the long-term production of seeds is not guaranteed.

4.3. Conservation Implications

The conservation of Aleppo pine is more than the conservation of a species: it includes conservation of a series of habitat types, and conservation of genetic diversity with associated evolutionary processes. The drought tolerance and relative fire tolerance of Aleppo pine suggests that its importance as a keystone species is only likely to increase in the face of climate change [42], [43]. However, the species is under threat from changes in land use, over-exploitation and invasive alien species - particularly novel pests and pathogens [44]. Moreover, since higher seed production of Aleppo pine is found at higher altitude, this further limits the availability of the most suitable habitat. This will produce a patchier distribution than predicted by suitable climatic conditions, as has been found with other Mediterranean pines [45].

Given the large physical separation from the nearest sizeable native populations (c. 450 km across the Mediterranean to Greece, c. 1 800 km overland to Tunisia), Libyan Aleppo pine likely represents an evolutionarily distinct population. Despite the forests being semi-natural at best, their preservation is even more important for long-term conservation. The widespread planting of Aleppo pine in Libya, both in the 20th century and potentially dating back to Classical times, may mean that evolutionarily important lineages are under threat [6]. Further, clearance, overgrazing and increased frequency of fire are leading to low population recruitment [14], hence reducing the ability of Aleppo pine to adapt to changing conditions. Conservation of genetic diversity in the species should therefore be a priority. However, while 34 Gene Conservation Units have been established for Aleppo pine in Europe, only four of these are from the eastern Mediterranean lineage and none are from North Africa. We therefore recommend the establishment of Gene Conservation Units, or equivalent, for stands of Aleppo pine in Libya.

These results suggest that to ensure continuation of Aleppo pine at the southern edge of its range, conservation efforts should be concentrated at the highest altitude since more seeds are produced there. Moreover, these seeds show better germination (Figures 3 and 4), produce longer roots (Figure 6) and higher root and shoot dry mass (Figure 7 and 8). These seedlings are thus most likely to grow better during spells of wet weather and be big enough to survive subsequent dry periods.

It is clear from the germination trials that total amount of water given is less important than the frequency at which it is given, particularly in terms of root growth [43]. It is likely that at higher altitude sites, the cooler minimum temperatures and higher precipitation compared to the lowest site studied (Table 1) will result in shorter periods of drought between rainfall events and thus increase seedling establishment and survival.

5. Conclusions

In conclusion, the study indicates that the remaining populations of Aleppo pine have a good future in northern Libya at least in the short-term since seed production is higher than the norm of more northern populations, and seedling growth is high even under low frequency of watering. Indeed, natural regeneration is currently expected to be high, particularly at higher altitudes in the Al Jabal Al Akhdar Mountain but this is likely to decline in the future. Further studies are needed on what conservation measures are possible in North Africa that would best benefit the long-term maintenance of these important populations in the face of climate becoming warmer and drier.

6. References

[1] Farjon, A. 2010. *A Handbook of the World's Conifers*. E.J. Brill, Leiden. Boston.

[2] Quézel, P. 1986. Les pins du groupement halepensis, végétation, ecophysiologie. *Option méditerannéennes. Série d'étude CIHEAM*, 1: 11-24.

[3] Richardson, D.M. and Nsikani, M.M. 2021. Mediterranean pines as invasive species in the Southern Hemisphere. Pines and their mixed forest ecosystems in the Mediterranean Basin, pp.83-99.

[4] Mauri, A., Di Leo, M., De Rigo, D. and Caudullo, G. 2016. *Pinus halepensis* and *Pinus brutia* in Europe: distribution, habitat, usage and threats. *European atlas of forest tree species*, pp.122-123.

[5] Bonari, G., Fernández-González, F., Çoban, S., Monteiro-Henriques, T., Bergmeier, E., Didukh, Y.P., Xystrakis, F., Angiolini, C., Chytrý, K., Acosta, A.T. and Agrillo, E. 2021. Classification of the Mediterranean lowland to submontane pine forest vegetation. *Applied Vegetation Science*, 24, e12544. doi.org/10.1111/ avsc.12544.

[6] Fady, B., H. Semerci and Vendramin, G.G. 2003. *EUFORGEN*

Technical Guidelines for genetic conservation and use for Aleppo pine (*Pinus halepensis*) and Brutia pine (*Pinus brutia*). International Plant Genetic Resources Institute, Rome, Italy.

[7] Euforgen 2023. http://www.euforgen.org/species/pinus-halepensis/ [Accessed 21 November 2024].

[8] Mechergui, K., Naghmouchi, S., Alsubeie, M.S., Jaouadi, W. and Ammari, Y. 2022. Biomass, radial growth and regeneration capacity of Aleppo pine, and its possible use as rootstock in arid and degraded areas. *iForest-Biogeosciences and Forestry*, 15(3):213-219.

[9] Guevara, J.C., Silva Colomer, J.H., Estevez, O.R. and Paez, J.A. 2003. Simulation of the economic feasibility of fodder shrub plantations as a supplement for goat production in the north-eastern plain of Mendoza, Argentina. *Journal of Arid Environment*, 53: 85-98.

[10] Reynolds, J.F. 2001. Desertification. In: Levin, S.A. (Ed.), *Encyclopedia of Biodiversity*, vol. 2. Academic Press, London, pp. 61–78.

[11] Quézel, P. 2000. Taxonomy and biogeography of Mediterranean pines (*Pinus halepensis* and *P. brutia*). In: Ne'eman G, Trabaud L (Ed.) *Ecology, biogeography and management of Pinus halepensis and P. brutia forest ecosystems in the Mediterranean basin*. Backhuys Publishers, Leiden, pp 1-12.

[12] *Global Forest Resources Assessment*. 2015. https://www.fao.org/3/i4793e/i4793e.pdf [Accessed 21 November 2024].

[13] Boulos, L. 1997. Endemic Flora of the Middle East and North Africa, in: H.N. Barakat, A.K. Hegazy (Ed.), *Reviews in Ecology, Desert Conservation and Development*, A festschrift for Prof. M. Kassas on the Occasion of 75th Birthday, IDRC/CRDI, UNESCO/South Valley University, Cairo, pp. 229-260.

[14] Hegazy, A.K., Boulos, L., Kabiel, H.F. and Sharashy, O.S. 2011. Vegetation and species altitudinal distribution in Al-Jabal Al-Akhdar landscape, Libya. *Pakistani Journal of Botany*, 43(4): 1885-1898.

[15] Mukassabi, T.A., Ahmidat, G., Sherif, I.M., Elmogasapi, A. Thomas P.A. 2012. Checklist and life forms of plant species in contrasting climatic zones of Libya. *Biological Diversity and Conservation*, 5(3): 1-12.

[16] Mahklouf, M.H. and Etayeb, K.S. 2018. Biodiversity in Libya. In *Global Biodiversity*. pp. 113-132. Apple Academic Press.

[17] Goubitz, S., Nathan, R., Roitemberg, R., Shmida, A. and Ne'eman G. 2004. Canopy seed bank structure in relation to: fie, tree size and density. *Plant Ecology*, 173: 191-201. doi: 10.1023/B:VEGE.0000029324.40801.74.

[18] Moya, D., Espelta, J.M., López-Serrano, F.R., Eugenio, M. and De Las Heras, J. 2008. Natural post-fire dynamics and serotiny in ten year-old Pinus halepensis Mill. stands along a geographic gradient. *International Journal of Wildland Fire*, 17: 287-292.

[19] Ayari, A., Moya, D., Rejeb, M.N., Ben Mansoura, A., Albouchi, A., De Las Heras, J., Fezzani, T. and Hanchi, B. 2011. Geographical variation on cone and seed production of natural Pinus halepensis Mill. forests in Tunisia. *Journal of Arid Environment*, 75: 403-410.

[20] Ayari, A., Zubizarreta, G.A., Tomé, M., Tomé, J., Garchi, S. and Henchi, B. 2012. Stand, tree and crown variables affecting cone crop and seed yield of Aleppo pine forests in different bioclimatic regions of Tunisia. *Forest Systems*, 21: 128-140. doi: 10.5424/fs/2112211-11463.

[21] Kanouni, M.R., Hani, I., Bousba, R., Beldjazia, A. and Khamar, H. 2020. Structural variability of Aleppo pine stands in two forests in northeastern Algeria. *Biodiversitas Journal of Biological Diversity*, 21(6):2848-2853.

[22] Turner, M.G., Turner, D.M., Romme, W.H. and Tinker, D.B. 2007. Cone production in young post-fire Pinus contorta stands in Greater Yellowstone (USA). *Forest Ecology and Management*, 242: 119-126.

[23] Vander Wall, S.B., 2023. Seed dispersal in pines (Pinus). *The Botanical Review*, 89(3):275-307.

[24] Harfouche, A., Boudjada, S., Chettah, H.W., Allam, M., Belhou, O. and Merazga, A. 2003. Variation and population structure in Aleppo pine (*Pinus halepensis* Mill) in Algeria. *Silvae Genet*, 52: 244-249.

[25] Nasri, N., Khaldi, A. and Triki, S. 2004. Variabilité morphologique des cônes et graines de Pin d'Alep et de Pin pignon en Tunisie. *Revue forestière française*, 56: 21-28.

[26] Climent, J., Prada, M., Calama, R., Chambel, R.M., De Ron, D.S. and Alia, R. 2008. To grow or to seed: ecotypic variation in reproductive allocation and cone production by young female Aleppo pine (*Pinus halepensis*, Pinaceae*). American Journal of Botany*, 95: 1-10.

[27] Malcolm, D.C., Mason, W.L. and Clarke G.C. 2001. The transformation of conifer forests in Britain regeneration, gap size and silvicultural systems. *Forest Ecology and Management*, 151: 7-23. doi: 10.1016/S0378-1127(00)00692-7.

[28] Girard, F., Vennetier, M., Guibal, F., Corona, C., Ouarmim, S. and Herrero A. 2012. *Pinus halepensis* Mill. crown development and fruiting declined with repeated drought in Mediterranean France. *European Journal of Forest Research*, 131: 919-931. doi: 10.1007/s10342-011-0565-6.

[29] Nathan, R., Safriel, U.N., Noy ‐ Meir, I. and Schiller, G. 1999. Seed release without fire in Pinus halepensis, a Mediterranean serotinous wind ‐ dispersed tree. *Journal of Ecology*, 87(4): 659-669.

[30] Messaoud, Y., Bergeron, Y. and Leduc, A. 2007. Ecological factors explaining the location of the boundary between mixed wood and coniferous bioclimatic zones in the boreal biome of eastern North America. *Global Ecology and Biogeography*. 16: 90-102.

[31] Climate Data. 2023. https://en.climatedata.org/africa/libya/jabal-alakhdar/massah%d9%85%d8%b3%d9%87-58012/#climate-table [Accessed 21 November 2024].

[32] Mukassabi, T. A., Polwart, A., Coleshaw, T. and Thomas, P. A. 2012. Scots pine seed dynamics on a waterlogged site. *Trees*, 26(4):1305-1315. doi: 10.1007/s00468-012-0706-7.

[33] Mukassabi, T. A., Polwart, A., Coleshaw, T. and Thomas, P. A., 2012. How long can young Scots pine seedlings survive waterlogging? *Trees*, 26(5):1641-1649. doi: 10.1007/s00468-012-0740-5

[34] Lepart, J. and Debussche, M. 1991. Invasion processes as related to succession and disturbance. In R. Groves and F. Di Castri (Ed.), *Biogeography of Mediterranean Invasions* (pp. 159-178). Cambridge: Cambridge University Press. doi:10.1017/CBO9780511525544.016.

[35] Mustafa, E., Tigabu, M., Aldahadha, A. and Li, M. 2024. Variations in cone and seed phenotypic traits among and within populations of Aleppo pine in Jordan. *New Forests*, 55(2):289-304.

[36] Nedjimi, B. and Guit, B. 2021. Salinity and temperature influencing seed germination of Mediterranean Aleppo pine (*Pinus halepensis* Mill.): an ecological adaptation to saline environments: Adaptation of *P. halepensis* to salinity and temperature. *Baltic Forestry*, 27(2):544:549.

[37] Henig-Sever, N., Eshel, A. and Ne'eman, G. 1996. pH and

osmotic potential of pine ash as post-fire germination inhibitors. *Physiologia Plantarum*, 96(1): 71-76.

[38] Mukassabi, T. A. 2011. *Scots pine (Pinus sylvestris L.) seed production at Wybunbury Moss, Cheshire and factors affecting seed germination and early establishment*. PhD thesis, Keele University. UK.

[39] IPCC 2023. *Climate Change 2023: Synthesis Report*. A Report of the Intergovernmental Panel on Climate Change. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Intergovernmental Panel on Climate Change, Geneva, Switzerland.

[40] Owens, J.N. and Blake, M.D. 1985. Forest tree seed production. A review of literature and recommendations for future research. Inf. Rep. PIX-53. *Canadian Forestry Service Petawawa*, ON.

[41] Rozas, V., Olano, J.M., Gazol, A., Alonso-Ponce, R., Cuende-Arribas, S. and Rodríguez-Puerta, F. 2024. Elevation and local climate variation control changes in Aleppo pine growth responses to hydroclimate and drought in semi-arid Spain. *Regional Environmental Change*, 24(2):87-95.

[42] Veuillen, L., Prévosto, B., Alfaro-Sánchez, R., Badeau, V., Battipaglia, G., Beguería, S., Bravo, F., Boivin, T., Camarero, J.J., Čufar, K. and Davi, H. 2023. Pre-and post-drought conditions drive resilience of *Pinus halepensis* across its distribution range. *Agricultural and Forest Meteorology*, 339:109577-109589.

[43] Feng, F., Wagner, Y., Klein, T. and Hochberg, U. 2023. Xylem resistance to cavitation increases during summer in *Pinus halepensis*. *Plant, Cell & Environment*, 46(6):1849-1859.

[44] Brondizio, E. S., Settele, J., Díaz, S. and Ngo, H. T. (Ed.) 2019. *Global Assessment Report on Biodiversity and Ecosystem Services of the Intergovernmental Science* - Policy Platform on Biodiversity and Ecosystem Services. IPBES Secretariat, Bonn.

[45] Bonari, G., Chytrý, K., Çoban, S. and Chytrý, M. 2020. Natural forests of *Pinus pinea* in western Turkey: a priority for conservation. *Biodiversity and Conservation*, 29: 3877-3898. doi.org/10.1007/s10531-020-02052-z.

7. Appendix:

Table 1. Tree and cone parameters for each of the six trees sampled at each site. Cone length and width and mean number of seeds/cone (n=50); mean mass of 100 seeds (n=10). ±SE.

Site	Tree no.	Tree height (m)	Trunk diameter (cm)	Age (y)	Number of cones	Cone length (cm)	Cone width (cm)	Mean Number of seeds/Cone	Mean of 100 seed mass (g)	Germination%*
A Albakour 181 a.s.l. (m)	1	7	22.3	35	53	10 ± 0.15	3.2 ± 0.14	77.8 ± 2.25	1.199 ± 0.0383	20.0[cd]
	2	8	20.4	32	68	7.4 ± 0.10	3.4 ± 0.03	57.6 ± 2.92	1.288 ± 0.0171	66.7[a]
	3	5	15.6	33	57	5.8 ± 0.09	2.7 ± 0.03	53.3 ± 3.17	1.279 ± 0.0217	10.0[d]
	4	4	14.0	36	217	6.0 ± 0.08	2.7 ± 0.03	57.3 ± 2.66	1.228 ± 0.0428	30.0[bcd]
	5	11	25.5	27	77	7.1 ± 0.90	2.6 ± 0.03	59.1 ± 2.87	1.467 ± 0.0338	50.0[ab]
	6	9	27.7	32	213	6.1 ± 0.09	2.6 ± 0.02	64.9 ± 3.12	1.200 ± 0.0004	46.7[abc]
B Alkouf 327 a.s.l (m)	1	11	25.8	24	118	9.2 ± 0.12	4.0 ± 0.08	90.8 ± 4.81	2.226 ± 0.0070	73.3[a]
	2	7	14.3	18	121	8.2 ± 0.20	2.8 ± 0.08	59.8 ± 3.22	2.664 ± 0.0083	30.0[b]
	3	8	12.1	17	113	8.0 ± 0.14	3.1 ± 0.04	101.5 ± 3.60	1.647 ± 0.0049	76.7[a]
	4	11	18.2	21	924	10 ± 1.15	3.2 ± 0.04	77.7 ± 2.25	1.883 ± 0.0046	30.0[b]
	5	9	14.0	20	53	9.5 ± 0.15	3.5 ± 0.03	54.9 ± 2.25	2.142 ± 0.0066	36.7[b]
	6	6	10.2	13	52	9.6 ± 0.11	3.9 ± 0.03	71.3 ± 2.40	1.728 ± 0.0146	36.7[b]
C Sidi Alhumry 843 a.s.l. (m)	1	16	26.0	32	1222	7.5 ± 0.13	3.5 ± 0.03	59.7 ± 2.49	2.594 ± 0.0101	76.7[a]
	2	14	25.5	43	1054	8.4 ± 0.09	3.6 ± 0.03	103.8 ± 2.09	1.904 ± 0.0219	86.7[a]
	3	15	21.5	43	1093	7.7 ± 0.09	4.1 ± 0.65	77.4 ± 1.95	2.295 ± 0.0198	53.3[a]
	4	11	19.5	28	897	10.1 ± 0.15	4.1 ± 0.05	81.0 ± 2.18	2.989 ± 0.0199	53.3[a]
	5	9	15.5	44	853	7.8 ± 0.09	3.7 ± 0.03	44.9 ± 2.37	2.641 ± 0.0279	70.0[a]
	6	14	24.5	39	991	8.9 ± 0.10	4.6 ± 0.04	95.4 ± 1.68	3.551 ± 0.0347	50.0[a]

*Different letters indicate significant differences.