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Effect of Seed Extraction Period and Germination Temperature on Viability of *Pinus patula* Under Controlled Conditions

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A number of coniferous species have demonstrated varied cone responses to the temperature intensities for seed release and subsequent seed germination behaviour. This study investigated the interactions of cone physical characteristics (weight and width), exposure duration (6,12 and 24 hours) at a fixed extraction temperature (65°C) and the germination temperature (22, 27 and 32°C) on seed quality of *Pinus patula*. The experimental design was a factorial experiment (4×3×3) laid down in a completely randomized design (CRD), with thirty-six treatments replicated 3 times. Analysis of the difference in means from the three factor effects from ANOVA was performed using R Statistical software. Where significant differences were observed, post hoc tests were carried out to separate means using the Tukey test at 5 % significance level. Results showed significant

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(*p*=0.001) differences in germination performance as a result of cone characteristics, extraction exposure periods, and germination chamber conditions. Seeds extracted from heavy cones and exposed to germination temperature of 32℃ demonstrated the highest germination percent at 90% while the lowest was 20% from light cones exposed to similar germination temperature conditions. Negative and significant correlation coefficients were observed in germination of seeds from narrow and light cones, thus cone sorting for heavy and wide cones was recommended for better germination performance of *P. patula* seed germplasm.

Keywords: Pinus patula; cone size; seed exposure; germination; correlation.

1. INTRODUCTION

Pine forests are the dominant vegetation in many parts of North America, Europe and Asia [1,2]. Pines exhibit a wide range of ecological strategies; some species fill early successional roles whereas others are late-successional trees [3,4]. Many other pine species are planted to revegetate disturbed sites, stabilize dunes, for shade and ornament, to provide windbreaks and for other purposes such as continuous supplies of raw materials for wood and wood-based industries [5,6]. Conifer plantations in all continents, and for the vast majority of regions, have demonstrated higher numbers of naturalized Pinaceae than those for Cupressaceae [7].

In some parts of Africa as elsewhere, the rate of growth of the indigenous tree species is low and incapable of meeting the growing demands for wood for the ever-increasing population. Therefore, plantations of fast-growing exotic tree species have been established to supplement the wood supplies from natural forests [8,9]. *Pinus patula* has been one of the most promising of the exotic coniferous species tried in many parts of Southern and Eastern Africa though success is restricted mainly to high altitude areas [10,11]. In Kenya, it is found on young fertile volcanic soils and on mature leached infertile soils. *P. patula* produces excellent fuel wood. The species is used in the commercial manufacture of pulp in the paper industry and its timber produces wood suitable for particle board manufacture and structural building construction [12–14]. In addition, when tapped, *P. patula* yields an oleoresin, which is distilled to give turpentine, and rosin which is used in painting and dyeing industries [15].

Fire disturbance has favoured pine expansion by facilitating cone bursting and stimulating germination especially for regenerations [16–19]. Fire intensity and frequency has acted as a factor of natural selection in pine species populations' regeneration and succession [20]. Pines in the absence of fire are poor in regenerating and often vulnerable to succession partly due to their serotinous nature as well as their inability to establish beneath their own canopy other than in small canopy gaps [21,22]. This explains the advantage that other species have over pine on similar sites, where there is a source of seed ready and available for the other forest species unlike the pines.

Higher temperature intensities (a function of the temperature reached and the time of exposure) has been shown to be a key factor in cone opening for seed release and also influences seed germination energy, rate and capacity [23–26]. Heat tolerance and thermal shock resistance as indicated by germination energy is shown to be dependent on species, cone and seed moisture content [10–12]. Thus, for extraction of seed in serotinous pines, the insulation capacity of cones and the thermal resistance of seeds, are believed to be the determinants for successful seed germination. Red pines have also shown variability in germination for cones that remained closed several years after fire. Initially the germination was low and slow for some cones but altogether, given time the germination increased to 70% at the end of the experiment [27].

It is known that pine populations can differ significantly in germination behaviour, for example, in germination percentage and time, prechilling requirements, or seed freezing tolerance [28,29]. The rate of seed extraction has been used to indicate the extraction efficiency in pines whereas the cone potential has been inferred to as a ratio between the number of germinable seeds vis a vis the total number of seeds in a cone [30,31]. A number of pine species especially in fire prone areas have previously been investigated for the insulation capacity of cones for safe seed release [20,26,32,33]. It was observed that under different treatments, their respective cone responses to the temperatures and subsequent seed germination varied across the species tested [34–36].

Germination responses under controlled conditions also exhibit some variance. In coniferous species, the level of cone protection against heat (insulation capacity) also varies from one species to another and thus is the seed protection from thermal insulation [37]. Studies by [38], have been carried out to assess the effects of forest fires to the regenerative abilities of pine stands after fire events especially in the Mediterranean where some pines were found to be fire tolerant as germination of seeds was not affected. Some studies have observed that seed germinability and responses to thermal shock greatly varied depending on the intensity and duration of exposure to thermal treatments [19,23].

In addition, for Aleppo pine, cone exposure to 30°C was determined to be safe for seed extraction as the procedure did not show any adverse effect on seed germination [39]. Lodgepole pine seeds heated to temperatures over 80℃ exhibited a significant decrease in germination suggesting existence of a threshold temperature above which germination is reduced. This threshold temperature coincided with the upper temperatures required to open serotinous cones. The study suggested that in terms of germination, seed from serotinous cones that were not heated above the threshold temperature could compete equally with seed from non-serotinous cones [40]. For *P. patula*, 65°C was earlier recommended (for rapid seed extraction as cones are set to open from 4 hours up to 24 hours. However, seed germinability was not tested, thus, the safe extraction exposure period was yet to be established. Therefore, the objectives of this study were: i) to analyze the correlations between cone width, exposure period and germination temperature on seed viability, and ii) to analyze the correlations between cone weight, exposure periods and germination temperature on seed viability.

2. MATERIALS AND METHODS

Cones were randomly collected from a seed orchard in Kamara block of Londiani forest, Kenya. Measurements of width and weight were taken by use of an electronic calliper and a KERN & Sohn (KB 10000-1N) balance respectively. The cones were categorised as either; narrow, wide, heavy or light depending on the width and weight, whereby the ranges used were; 20g to 27g for light, 28g to 34g for heavy, 2.5cm to 2.8cm for narrow and 2.9cm to 3.2cm for wide cones respectively [41]. These cones were then exposed to artificial extraction conditions in ovens set at 65℃ [42]. Observations of the seed released were done after 6, 12, and 24 hours. Extracted seed was cleaned by removing the empty wings and dewinging the full seeds. Each petri dish had 100 seeds replicated three times from each of the extraction treatments [43]. Radicle emergence was used as the criterion for germinability [38,44]. The number of germinated seeds were recorded daily for up to day 28 with those counted being discarded (counting without replacement). Observations of germination was indicative of seed vigour for seeds exposed to the different controlled conditions.

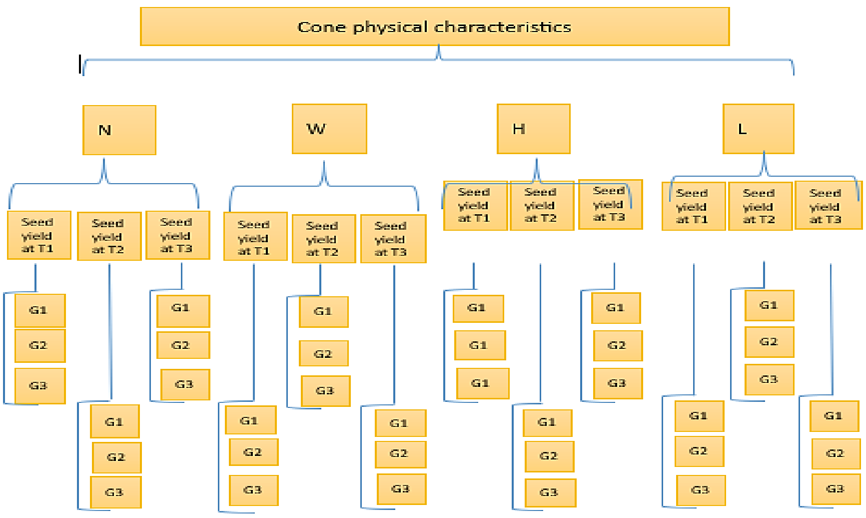

Fig. 1. Sketch of study design

The experiment consisted of 36 treatments replicated 3 times with the following as treatments:

1. Cone characteristics: H= Heavy weight; L= Light weight; W= Wide cone, N= Narrow cone
2. Duration of exposures for seed extraction: Exposure T1= 6 hours, Exposure T2= 12 hours, Exposure T3= 24 hours.
3. Germination chamber temperature: G1=22 °C, G2=27 °C, and G3=32 °C

The study design (Fig. 1) is illustrated as follows:

3. RESULTS

In the category of cones grouped on basis of cone width, seed extraction periods showed weak negative relationships with germination performance in seeds extracted from narrow cones (Table 1). Similarly, germination temperature also showed weak negative but significant relationships with germination performance in this narrow cone category.

In this (narrow) cone category germination trends, the highest germination percentage (69.0%) was observed from seeds extracted in the first six hours and exposed to 32℃ for germination and the least (25.5%) was observed from seeds of the last twelve hours of extraction exposed to a germination temperature of 22℃ (Fig. 2).

Analysis of correlations in seed germination performance of seeds from the wide cones under the varied extraction periods and different germination temperature conditions showed some positive relationships but no significant relationships between germination success and germination temperature (Table 2).

There were insignificant differences in germination in the three chamber temperatures as demonstrated in the germination trends. On the other hand, there were significant differences in germination performance of seeds from wide cones in relation to the extraction exposure periods (F=16.415 (F_{crit}=4.757), p=0.0027). The seeds which showed better germination performance at the three chamber temperatures in the wide cones category were those exposed to extraction for 24 hours without breaks (Fig. 2).

Cone weight and extraction exposure periods generally showed a negative relationship with germination. Germination performance negatively interacted with both germination temperatures and extraction exposure periods when cone weight was considered.

Moderately strong negative and significant correlation was observed in seed germination performance from seeds of light cones with effects from extraction periods (Table 3). Germination performance and germination temperature correlated positively, weakly and insignificant.

Seeds exposed to the three extraction periods from heavy cones showed weak but positive interaction with germination performance (Table 4). This weak positive correlation was not statistically significant. For this group the germination performance negatively correlated with germination temperature conditions, although not significant.

Table 1. Pearson correlation coefficient for germination of seeds from narrow cones

Variables	Germination temperature	Extraction period	Germination performance
Germination temperature	1		
Extraction period	.100ns	1	
Germination performance	-.244**	-.323**	1

*** Correlation is significant at the p≤0.05 level. ns: p>0.05 correlation not significant*

Table 2. Pearson correlation coefficient for germination of seeds from wide cones

Variables	Germination temperature	Extraction period	Germination performance
Germination temperature	1		
Extraction period	-.085ns	1	
Germination performance	.086ns	-.128ns	1

ns: p>0.05 correlation not significant

Table 3. Pearson correlation coefficient for germination of seeds from light cones

Variables	Germination temperature	Extraction period	Germination performance
Germination temperature	1		
Extraction period	.007	1	
Germination performance	.085	-.618**	1

***. Correlation is significant at the p≤ 0.05 level; ns: p>0.05 correlation not significant*

Table 4. Pearson correlation coefficient for germination of seeds from heavy cones

Variable	Germination temperature	Extraction period	Germination performance
Germination temperature	1		
Extraction period	-.004ns	1	
Germination performance	-.034ns	.089ns	1

ns: p>0.05 correlation not significant

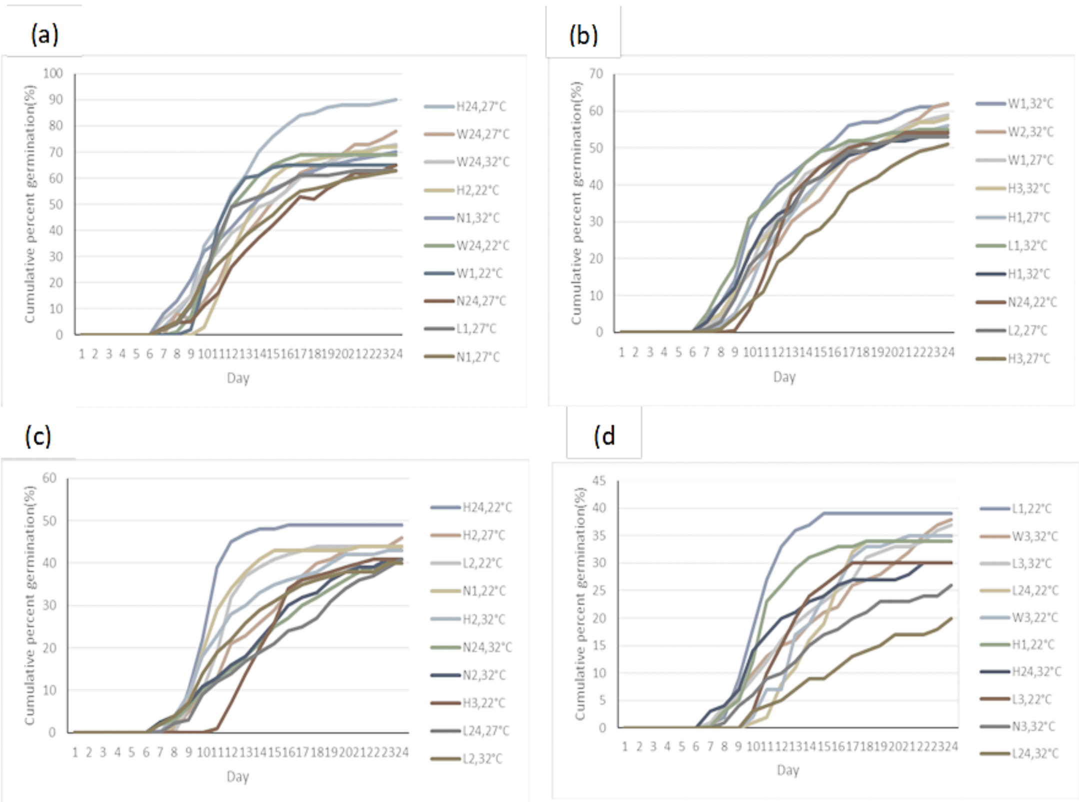

Fig. 2. Germination trends based on different seed extraction and germination conditions

Under controlled conditions, germination was observed to begin from 7 to 9 days. Seed extracted from heavy (90%) and wide (80%) cones after 24 hours, generally showed better germination performance cumulatively under 27℃ chamber conditions. Seeds from light cones subjected to 24 hours exposure for extraction and set at 32℃ chamber conditions had the least cummulative germination percent 26.3%. The early radicle emergers were observed at 32 and 27℃ chamber temperatures with late risers at 22℃.

Germination performances analysed by ANOVA for seed samples from heavy cones extracted in phases and also in 24 hours without breaks showed no significant differences in germination performance of seeds germinated under the three chamber conditions. There were also no significant differences in germination resulting from the exposure periods. This indicates that differences in germination of seeds from the heavy cones group were not significant either with extraction period or with germination chamber temperature as a factor. A similar performance was experienced in seeds from light cones.

The relationships between the cone characteristics: width and weight, extraction exposure periods and germination gave the following regression equations:

$G_{width} = 40.7 + 0.700_{gt} - 0.913_{ex}$ with R^2=87.7% and $G_{weight} = 35.5 + 0.500_{gt} - 0.238_{ex}$ with R^2= 7.3%.

Where; G=germination performance (%), ex= extraction period (hours), gt= germination temperature (℃).

4. DISCUSSION

Variations in seed germination performance has been noted in the results of the current study with differences in cone traits and changes in extraction exposure period and germination. Within the groups, based on extraction period the first phase of extraction, twelve hours showed positive influence on germination potential in the three chamber conditions. However, there was steady decline of germination with progression in extraction phases in groups L and N (seeds from light and narrow cones). This showed that as the extraction time increased, the number of seeds germinated reduced at warmer chamber temperatures.

The trends in the narrow cones category showed that germination sensitivity was affected by seed extraction exposure periods as increased exposure periods within the phases of extraction had declining germination performance. The variations in germination exhibited based on the cone width characteristic showed much sensitivity to exposure periods of extraction and temperature intolerance by how wide or narrow a cone is. Narrow cones exhibited much intolerance to temperature variations, (cooling within breaks) as extracted seeds showed more deterioration with changes or increase in exposure periods and germination temperature. These findings agrees with earlier work by [20,45].

This study focused on the germination capacity as post germination performance which have been shown to even out in the field over time as the seedlings get established. Germination performance has been used to characterize the physiological quality of pine seeds as documented by [46]. Several parameters have been used to characterize germination performance of pine; lodgepole pine (*Pinus contorta)* seed lots. These include; germination the time of germination onset (lag), germination speed (rate), and extent or capacity (cumulative germination percentage at the end of the testing period). Further investigations showed that germination capacity (% germination) was the most important parameter in determining the suitability of a seed lot for commercial use, but germination rate influences the uniformity of emergence in nurseries as earlier documented by [47–49].

Previous studies have shown that both environmental and genetic factors influence the potential of trees to produce fertile quality seeds whereby depending on the tree species, seed germination varied according to latitude, elevation, soil moisture, soil nutrient, temperature, type and density of plant cover, and degree of habitat disturbance of the site [49,50]. Location of seed at the time of maturity plays an important role in determining a forest tree's potential for seed quality and seed fertility [51–53].

Other studies have shown instances where there is positive correlation between the extraction exposure period, cone productivity and germination. In these studies, cone and seed germination traits were shown to be genetically inclined [28]. In an effort to understand the effects of cone size and seed mass on recruitment of maritime pine, results demonstrated that, although seed weight was suggested to be an indicator of robust seedlings, heavier cones tended to generate more mature seeds, but not necessarily heavier ones. Overall, according to the study, for maritime pines, in as much as seed mass was an indicator for seedling growth, seedling viability was not compromised by seed mass. A positive relationship was found between seed size and seedling growth pointing that larger seeds were more likely to survive after heat shocks [54]. This recent finding corroborates with the current study.

Seeds extracted from cones in the light category at the end of the 24 hours without breaks, had the lowest germination performance while the best performance was from heavy cones within the same germination condition (32℃). The results of this study showed that germination rates decline with increase in exposure period within the three phases of extraction. Earlier studies by [24] showed that exposure to higher seed extraction temperatures negatively influence germination of pine seeds.

Seeds from heavy cones exhibited lower seed moisture indicating fuller embryo thus little space for water. Lower seed moisture content reduces the risk of thermal shock depending on the germination temperature conditions [4]. Additionally, heavy cones could be expected to have better insulating properties protecting the seeds from deteriorating when exposed for longer periods. As a result of these differences in cone characteristics and exposure duration, seeds responses to thermal shocks were also different. Moisture content has been shown to affect seed deterioration rate and has impacted on storage longevity of seeds as earlier documented by [55,56]. Thus, drying of seeds has been known to increase the storage life. Warmer temperatures were demonstrated to have an overall better germination which dropped with lower temperatures.

Whereas wide cones yielded the most seed, the highest germination was observed from seeds of heavy cones. A comparison of the results shows that increasing seed exposure duration phases at the constant temperature of 65℃ increased in the percentage of seed germination and is lower in lighter cones. Insulation capacities of cones have been shown to differ between species and within pine species with other species such *P. halepensis* shown to be more resistant to higher temperatures, whereas *P. nigra* and *P. sylvestris* experienced inhibited seed germination at higher temperatures [23,32,57].

Seed germination is shown to deteriorate more when extracted in phases or in breaks than when exposed to a full 6 hours or an entire 24 hours. The breaks could indicate brief changes in temperature and humidity after the beginning of cone opening. This shows that the extraction conditions, seed sensitivity and tolerance, during seed handling also influences seed germination behaviour. Constant extraction conditions positively influenced the seed germination.

The mean germination and early survival did not differ significantly between the groups selected samples germinated under open nursery conditions. Seeds from narrow cones extracted in the second phase had the lowest survival. Extended drying periods did not significantly lead to decreased seed quality under nursery conditions as the varied exposure duration effect somehow evened out during the seedling early survival.

Notably, germination seemed to decline for seeds extracted in day 11 and 12. Seeds extracted within the first 3 to 7 days under drying bed conditions exhibited better germination performance. Germination vigour observed early and late radicle emergers in comparison had similar overall performance. As expected, germination temperature conditions played a crucial role in initiating germination. An earlier study had pointed out temperature among other factor as most influential in germination rate and synchrony [58].

5. CONCLUSION

P. patula cone weight showed greater influence on germination than cone width. Seeds produced from heavier cones gave better germination performance than those from wide, light and narrow cones. Bigger cones have demonstrated better capacity in producing fuller seeds, protective (insulation) abilities and overall elasticity to environmental changes. Thus, these properties by extension, encourage development and production of sound, robust, equally resilient seeds. Seed germination conditions had greater influence on germination than the extraction exposure durations and cone characteristics. This was shown in the higher seed germination performance trends at 32℃. This could be explained as increased seed vigour with warmer temperatures. Radicle emergence and physiological process during germination seems to be favoured by warm temperature as low germination temperatures give a chilling effect thereby increasing the inhibition mechanisms of seeds.

6. RECOMMENDATIONS

Germination under controlled temperatures showed consistency, hence recommendation of warmer temperatures between 27℃ to 32℃ for higher germination performance. To enhance the quality of germplasm resources, cone sorting of *Pinus patula* for wider and heavier cones is recommended for improved seed extraction and germination potential.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Payn T, Carnus JM, Freer-Smith P, Kimberley M, Kollert W, Liu S et al. Changes in planted forests and future global implications. For Ecol Manage. 2015;352:57–67. Availablefrom:http://dx.doi.org/10.1016/j.foreco.2015.06.021
2. Bialobok S, Zelawski W. Outline of physiology of Scots pine. 1976;Vol. TT 73-5408:365.
3. Martínez-Ramos M, Pingarroni A, Rodríguez-Velázquez J, Toledo-Chelala L, Zermeño-Hernández I, Bongers F. Natural forest regeneration and ecological restoration in human-modified tropical landscapes. Biotropica. 2016;48(6):745–57.
4. Barbour MG. Closed-cone pine and cypress forests. In: Barbour MG, Keeler-Wolf T, Schoenherr AA, editors. Terrestial vegetation of California. Third. Los Angeles: University of California Press. 2007;296–312. Availablefrom:https://www.degruyter.com/document/doi/10.1525/9780520933361-013/html
5. Richardson DM, Williams PA, Hobbs RJ. Pine invasions in the southern hemisphere: Determinants of spread and invadability. J Biogeogr. 1994;21(5):511.
6. Ramírez-Mosqueda MA, Iglesias-Andreu LG, Armas-Silva AA, Cruz-Gutiérrez EJ, de la Torre-Sánchez JF, Leyva-Ovalle OR et al. Effect of the thin cell layer technique in the induction of somatic embryos in *Pinus patula* schl. et Cham. J For Res. 2018;30(4):1535–9.
7. Essl F, Mang T, Dullinger S, Moser D, Hulme PE. Macroecological drivers of alien conifer naturalizations worldwide. Ecography (Cop). 2011;34(6):1076–84.
8. Köhl M, Lasco R, Cifuentes M, Jonsson Ö, Korhonen KT, Mundhenk P et al. Changes in forest production, biomass and carbon: Results from the 2015 UN FAO Global Forest Resource Assessment. For Ecol Manage. 2015;352:21–34. Availablefrom:http://dx.doi.org/10.1016/j.foreco.2015.05.036
9. Keenan RJ, Reams GA, Achard F, de Freitas JV, Grainger A, Lindquist E. Dynamics of global forest area: Results from the FAO Global Forest Resources Assessment 2015. For Ecol Manage. 2015;352:9–20. Availablefrom:http://dx.doi.org/10.1016/j.foreco.2015.06.014
10. Adegbehin JO. Growth and yields of *Pinus patula* in some parts of eastern Africa with particular reference to sao-hill, southern Tanzania. Commonw For Rev. 2016; 61(1):27–32. Availablefrom:http://www.jstor.org/stable/42606126
11. Crous JW, Morris AR, Scholes MC. Effect of phosphorus and potassium fertiliser on stem form, basic wood density and stem nutrient content of *Pinus patula* at various stem heights. Aust For. 2009;72(2):99–111.
12. Kuria NC, Balozi KB, Kipkore W. Growth and yield models for plantation-grown cupressus lusitanica for central Kenya. African J Educ Sci Technol. 2019;5(2):34–58. Availablefrom:http://ajest.info/index.php/ajest/article/view/351
13. Ngugi MR, Mason EG, Whyte AGD. New growth models for Cupressus lusitanica and *Pinus patula* in Kenya. J Trop For Sci. 2000;12(3):524–41.
14. Brister G, Fry G. Kenya pine timber — Structure and strength (O. D. C .8). East African Agric For J. 1966;31(3):343–8.
15. Orwa C, A Mutua, Kindt R, Jamnadass R SA. *Pinus patula Pinus patula* Schldl. et Cham. Agroforestree Database: A Tree Reference and Selection Guide Version 4.0. 2009;Vol. 0:1–5.
16. Tapias R, Gil L, Fuentes-Utrilla P, Pardos JA. Canopy seed banks in Mediterranean pines of southeastern Spain: A comparison between *pinus halepensis* Mill, P pinaster

Ait, P nigra Arn and P pinea L. J Ecol. 2001;89(4):629–38.

17. Ganatsas P, Tsakaldimi M, Thanos C. Seed and cone diversity and seed germination of *Pinus pinea* in strofylia site of the natura 2000 network. Biodivers Conserv. 2008, Sep 18;17(10):2427–39. Availablefrom:http://link.springer.com/10.1007/s10531-008-9390-8
18. Tapias R, Climent J, Pardos JA, Gil L. Life histories of mediterranean pines. Plant Ecol. 2016;171(1):53–68.
19. Fillingim H, Knapp BO, Kabrick JM, Stambaugh MC, Elliott GP, Dey DC. Direct and indirect effects of fire on germination of shortleaf pine seeds. Fire Ecol. 2023;19(53). Availablefrom:https://doi.org/10.1186/s42408-023-00214-2
20. Koba VP, Zhigalova TP. Experimental assessment of postfire resilience of pallasian pine seeds. Contemp Probl Ecol. 2019;12(7):724–30.
21. Kosanke RM. Demographic processes underpinning post-fire resilience in California closed-cone pine forests: The importance of fire interval, stand structure, and climate. 2019;1–42. Available from: https://www.researchsquare.com/article/rs-913774/v1.pdf
22. Haymes KL, Fox GA. Variation among individuals in cone production in pinus palustris (PINACEAE). Am J Bot. 2012;99(4):640–5.
23. Lovreglio R, Salvatore R, Giaquinto P, Leone V. Thermal treatments and germination Response over time of seeds from serotinous and non serotinous cones of *pinus halepensis* Mill. In: International Workshop MEDPINE 3: Conservation, Regeneration and Restoration of Mediterranean Pines and Their Ecosystems. 2007;155–66.
24. Aniszewska M, Gendek A, Tulska E, Peska P, Moskalik T. Influence of the duration of microwave irradiation of scots pine (*pinus sylvestris* L.) cones on the quality of harvested seeds. Forests. 2019;10(12):1–15.
25. Aniszewska M, Zychowicz W, Gendek A. The effectiveness of short-term microwave irradiation on the process of seed extraction from Scots pine cones (*Pinus sylvestris* L.). iForest - Biogeosciences For. 2020, Feb 29;13(1):73–9. [cited 2020 Apr 23]. Availablefrom:http://www.sisef.it/iforest/contents/?id=ifor3089-012
26. Calvo L, Nunez MR. Effect of high temperatures on seed germination of *Pinus sylvestris* and Pinus halepensis. For Ecol Manage. 2000;131:183–90.
27. Çetin B. The effect of altitude and closed cone (seed) age on germinationin Red Pine (*Pinus brutia* Ten.). Sumar List. 2023;147(3–4):129–35.
28. Correia I, Santos L, Faria C, No C, Almeida H, David T. Cone to seedling—Variation between. For Sci. 2014; 60(4):724–32.
29. Tonguç M, Önder S, Gülcemal N, Tonguç F. Seed, germination, and seed-reserve traits differ along an altitudinal gradient. J For Res. 2022;33(6):1903–12. Availablefrom:https://doi.org/10.1007/s11676-022-01467-4
30. Grayson KJ, Wittwer RF, Shelton MG. Cone characteristics and seed quality 10years after an even-aged regeneration cut in Shortleaf Pine stands. In: Proceedings of the eleventh biennial southern silvicultural research conference. Ashville. 2002;310–4.
31. Bramlett DL. Efficiency of seed production in southern. In: Thirteenth lake states forest tree improvement conference. U.S. Department of Agriculture, Forest Service; 1977. Availablefrom:https://www.fs.usda.gov/treesearch/pubs/14783
32. Habrouk A, Retana J, Espelta JM. Role of heat tolerance and cone protection of seeds in the response of three pine species to wildfires. Plant Ecol. 1999;145(1):91–9.
33. Moya D, Espelta JM, Verkaik I, López-Serrano F, De Las Heras J. Tree density and site quality influence on *Pinus halepensis* Mill. reproductive characteristics after large fires. Ann For Sci. 2007;64(6):649–56.
34. Wyse SV, Brown JE, Hulme PE. Seed release by a serotinous pine in the absence of fire: Implications for invasion into temperate regions. AoB Plants. 2019;11(6):1–8.
35. Bhat GM, Mughal AH, Malik AR, Islam MA. Chemical science review and letters developing a new technique for rapid seed extraction from the cones of himalayan conifer (*Pinus wallichiana* A.B. Jackson). Chem Sci Rev Lett. 2017;6(22):746–51.

36. Sivacioğlu A, Ayan S. Evaluation of seed production of scots pine (*Pinus sylvestris* L.) clonal seed orchard with cone analysis method. African J Biotechnol. 2008; 7(24):4393–9.
37. Reyes O, Casal M. Effect of high temperatures on cone opening and on the release and viability of *Pinus pinaster* and P. radiata seeds in NW Spain Otilia. Ann For Sci. 2001;64:219–28.
38. Ghildiyal S, Sharma C, Gairola S. Effect of temperature on cone bursting, seed extraction and germination in five provenances of *Pinus roxburghii* from garhwal Himalaya in India. South For a J For Sci. 2008, Jul;70(1):1–5. Availablefrom:http://www.tandfonline.com/doi/abs/10.2989/SOUTH.FOR.2008.70.1.1.511
39. Singh A, Husain M, Mir RA, Parrey AA, Paddar NA. Effect of different seed extraction methods for rapid extraction of seeds from the cones of Aleppo pine (*Pinus halepensis*) under controlled conditions in kashmir valley, India. Pharmacogn Phytochem. 2017;6(4):1622–5.
40. Knapp AK, Anderson JE. Effect of heat on germination of seeds from serotinous lodgepole pine cones. Am Midl Nat. 1980;104(2):370–2.
41. Angaine PM, Onyango AA, Owino JO. Morphometrics of *Pinus patula* crown and its effect on cone characteristics and seed yield in Kenya. J Hortic For. 2020, Jul 31;12(3):94–100. Availablefrom:https://academicjournals.org/journal/JHF/article-abstract/EA3616F64129
42. Onyango AA, Angaine PM, Inoti SK, Owino JO. Patula pine (*Pinus patula*) cones opening under different treatments for rapid seed extraction in londiani, Kenya. J Hortic For. 2020, june;12:63–9.
43. ISTA. International rules for seed testing. Int rules seed Test. 2023;12:345. (International rules for seed testing, Zurich, Switzerland.).
44. ISTA. ISTA News Bulletin: Towards automated single counts of radicle emergence to predict seed and seedling vigour. Seed Test Int. 2011;(142):44–8.
45. Bae H, Kim J. Functional principles of morphological and anatomical structures in pinecones. Plants. 2020;9(1343):1–11.
46. Novikov AI, Zolnikov VK, Novikova TP. Grading of scots pine seeds by the seed coat color: How to optimize the engineering parameters of the mobile optoelectronic device. Inventions. 2021; 6(1):1–11.
47. Bacherikov IV, Raupova DE, Durova AS, Bragin VD, Petrishchev EP, Novikov AI et al. Coat colour grading of the scots pine seeds collected from faraway provenances reveals a different germination effect. Seeds. 2022;1(1):49–73.
48. El-Kassaby YA, Moss I, Kolotelo D, Stoehr M. Seed germination: Mathematical representation and parameters extraction. For Sci. 2008;54(2):220–7. Availablefrom:https://academic.oup.com/forestscience/article/54/2/220/4604412
49. Yousefpour R, Temperli C, Jacobsen JB, Thorsen BJ, Meilby H, Lexer MJ et al. A framework for modeling adaptive forest management and decision making under climate change. Ecol Soc. 2017, Dec 1;22(4).
50. Batkhuu N-O, Udval B, Jigjid B-E, Jamyansuren S, Fischer M. Seed and cone morphological variation and seed germination characteristics of scots pine populations (*Pinus sylvestris* L.) in Mongolia. Mong J Biol Sci. 2020;18(2):41–5.
51. Beckman NG, Aslan CE, Rogers HS, Kogan O, Bronstein JL, Bullock JM et al. Advancing an interdisciplinary framework to study seed dispersal ecology. AoB Plants. 2020;12(2):1–18.
52. Guemri R El, Jaouadi W, Mechergui K, Alsubeie M, Naghmouchi S, Ouellani S El et al. Morphological characteristics and variation of wood, cone and seed productions in the reforestation of aleppo pine in northeastern tunisia using terrestrial and spatial index approaches. Ekol Bratislava. 2019; 38(3):273–91.
53. Zhang Y, Hu P, Zhong C, Wei Y, Meng J, Li Z, et al. Analyses of genetic diversity, differentiation and geographic origin of natural provenances and land races of Casuarina equisetifolia based on EST-SSR markers. Forests. 2020, Apr 10;11(4):432. Availablefrom:www.mdpi.com/journal/forests
54. Maia P, Corticeiro S. From cone to seed and seedling—Characterization of three portuguese *Pinus pinaster* Aiton Populations. Seeds. 2022;1(2):126–35.
55. Tangney R, Merritt DJ, Fontaine JB, Miller BP. Seed moisture content as a

primary trait regulating the lethal temperature thresholds of seeds. J Ecol. 2019; 107(3): 1093–105.
56. Karrfalt RP. Seed moisture content, relative humidity, and better storage of longleaf pine seed. Tree Plant Notes. 2017;60(2):63–9.
57. Lamont BB, Pausas JG, He T, Witkowski ETF, Hanley ME. Fire as a selective agent for both serotiny and nonserotiny over space and time. CRC Crit Rev Plant Sci. 2020;39(2):140–72. Availablefrom:https://doi.org/10.1080/07352689.2020.1768465
58. Bravo-Navas MV, Sánchez-Romero C. Germination behavior and early seedling growth in abies pinsapo Boiss. Seeds. Plants. 2022;11(20):1–12.

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