

## **Influence of Cone Physical Characteristics and Extraction Exposure Period on Seed Yield of *Pinus patula***

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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**

This study examines the correlation between extraction exposure periods and cone physical characteristics on *Pinus patula* seed yield. Systematic random sampling was employed for tree identification in an even-aged clonal seed orchard, and the laboratory phase was laid down as a factorial experiment with two factors: cone physical characteristics and extraction exposure period at three levels. Seed counts were taken for cones categorized as; heavy, light, narrow, and wide at three extraction exposure periods 6 hours, 12 hours, and 24 hours in a constant oven temperature of 65°C. The experiment had 12 (L1,H1,N1,W1,L2,H2,N2,W2,L3,H3,N3,W3) treatments with 60 cones per treatment. The time spent counting and returning cones during the inter-stage observation ranged from 10 to 15 minutes. Data collected were tabulated and means analyzed using ANOVA with results generated as per the objectives. The number of seeds released within the hours of exposure was captured as the seed extraction rate. The first six hours yielded the optimum number of seeds per cone with the mean highest number of seeds from wide cones. The lowest mean number of seeds released observed was 28, from light cones, while the highest mean number of seeds was observed to be 56 from wide cones. Cone sorting based on size before

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extraction is recommended for optimized seed yield. The stages of seed extraction employed here can be used in mechanized seed extraction cabinets equipped with timers at controlled temperatures.

**Keywords:** *Pinus patula*; cone characteristics; extraction; seed yield.

## 1. INTRODUCTION

Pines are one of the fast-growing exotic tree species established for commercial plantation development in East and Southern Africa [1,2]. The bulk of seed extraction is concentrated in pine cones due to the high demand for pine seed [3–6]. *Pinus patula* is a serotinous pine and thus produces serotinous cones [7–9]. Serotinous cones retain their seeds for one or more years following maturity [10–13] with seed release often occurring in response to an environmental stimulus such as high temperature [13,14].

Seeds extraction from cones of many species requires drying either natural or artificial heat sources and several methods have been employed for seed extraction from such cones [3,15–17]. Challenges in seed extraction from cones have been attributed to scale opening and deflection which has been observed to be changing between coniferous species and also cone parts. The middle and top parts show significant deflection in some species while the next-to-stem part does not change its position significantly [3]. *P. patula* is one of the species whose cone opening has demonstrated more reliance on cone width than cone length as there is little or no deflection on the next-to-stem [17]. Silviculture focused on optimizing the seed extraction process is expected to enhance species seed production and preservation [18]. The control of energy use in the process of seed extraction is important to ensure sustainable development while promoting the conservation of natural resources [19]. The seed extraction process has been used in wet and humid pine-growing countries for the controlled production of seed from silvicultural extraction facilities [20]. Conifers have displayed between-species and within-species cone differences in terms of cone morphology, density, mass, weight, color, degrees of asymmetry, differences in scale tension, the effect of environment on bonding strength, and genetic differences in bonding agent [21,22].

The mechanism of scales opening in *Pinus* genera has also been proved to experience significant differences longitudinally (lengthwise),

than cross-sectionally through shrinkage from wet to dry [23]. Whereas cone length, diameter, and volume do not vary significantly by stand density, tree diameter, or crown position, cone dry weight differs significantly between released and unreleased crop trees. The number of potentially productive scales per cone also varies significantly by location within the crown with greater numbers in the upper crown. Cone orientation, based on uprightness in *P. strobiformis* has also been reported to have no significant effects on seed extraction rates calculated as the number of seeds released per second but there existed cone opening differences over different times of the year [24]. Information on cones and seeds has been found as effective guides for breeders when evaluating seed production from seed orchards [25].

A few pines have worked on examining the ideal conditions for seed release from serotinous cones and a variety of methodological approaches have been applied where these conditions have been examined. Typical approaches focus on the temperatures required to release the scales of serotinous cones and involve either heating cones in ovens or immersion in water [11,12,26]. Previous studies have reported that pine cones open to release seed through a combination of temperature and humidity which varies widely in the pine-growing regions of the world [13,27,28]. Another factor affecting pine cone opening is the presence of resin which occurs in many species [13]. In these species, cone scales remain closed due to sealing with resin which requires high temperatures for resins to melt and cones to open. An increase in temperature which was comparable to longer exposure periods has been shown to affect seed release [29].

Artificial drying of cones in heated kilns has been recommended for cool moist climate species where the climate is not suited for air drying [5,30]. In Kenya, cones are sun-dried in open-sided sheds covered with plastic roofs, where they open after 2 to 14 days. The duration of cone opening is long due to the low temperatures in pine seed extraction regions. *Pinus patula* is a priority commercial tree species in Kenya, and

therefore requires the availability of information on seed extraction efficiency. The study seeks to rove on the extraction practice by the use of empirical data to demonstrate the efficiency of artificial seed extraction technique that shortens the extraction period and optimize seed release. This will further enhance the planning processes for seed collection and production, especially for germplasm producers. The findings of this study will improve the existing knowledge and procedures of seed extraction from cones by providing new insights into the process.

The objectives of this study therefore were:

- i) to assess the correlations of cone width and exposure period on seed release and ii) to assess the correlations of cone weight and exposure period on seed release

## 2. MATERIALS AND METHODS

### 2.1 Stand Description and Tree Selection

A 2006 *Pinus patula* clonal seed orchard stand was used as the source of trees and cones for this study. The seed source is situated in the Kamara block of Londiani forest, Kenya. The spacing used during establishment was 5m by 5m and the stand was pollarded in 2019. The stand has a mean height of 26.5m. Forty-five trees were systematically selected with a random start. The trees were marked and using a diameter tape, the diameter at 1.3 meters above ground, referred to as the diameter at breast height or DBH of each sampled tree was measured to examine whether the cone characteristics of each tree are correlated with tree size. Londiani area is cool and moist most of the year and is at an elevation of 2,308 m asl. The average temperatures during the time of this study ranged between 10.5°C and 25°C. The area has two rainy seasons, long rains occurring in the months of March to May with an average rainfall of 750 mm for the three months, and short rains in October to December with average rainfall for the three months of 423 mm. The driest months are January to February and August to September [31].

### 2.2 Cone Collection

At the time of cone collection (June 2022), four of the selected trees had no mature cones while others had sufficient numbers thus the number of cones collected per tree ranged from 11 to 63

making a total of 1980 cones collected. Given that cone, maturity age has been found to influence opening temperature in some species, such as *P. halepensis* [11], observation of cone color (shiny brown to silver grey) was the criteria for mature cones of the current season. The cones were packed in gunny bags for delivery to the KEFRI Londiani laboratory situated at 35.607270°E longitude and 0.155520°S latitude.

Once in the laboratory, measurements of width and weight were taken by use of an electronic caliper and a KERN & Sohn (KB 10000-1N) balance. The cones were categorized 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 [32]. These cones were then exposed to artificial extraction conditions in ovens set at 65°C [17]. Observations of the seed released were done after 6 hours, 12 hours, and 24 hours. The categories under investigation were; Light cones, Heavy cones, Narrow cones, and Wide cones denoted as L, H, N, and W respectively with alphanumeric 1,2,3 representing extraction exposure periods; 6 hours, 12 hours, and 24 hours. Whereas exposure periods and cone characteristics were treatments, the variable was the seed released within the periods. The examined cases of seed extraction were compared in terms of cone weight and width, and the quantity of seed obtained at the extraction exposure times. The conventional way of seed drying in beds was the control from which daily observations were made for up to 21 days. A set of 30 cones from each of the categories (light, heavy, wide, and narrow) were set in Petri dishes and placed outside under the sun, and observed for seed release.

Descriptive statistics were applied to study the cone composition within each category. The variables and factors were subjected to a one-way analysis of variance (ANOVA) to determine the differences between and within groups. Tukey's HSD (Honestly Significant Difference) was used to find significant differences in means between groups at  $p=0.05$ . The correlations between the cones' physical attributes and the diameter of the tree were determined by the linear regression method to account for the documented relationships between tree size and cone traits [33–36].

### 3. RESULTS AND DISCUSSION

#### 3.1 Tree Diameter and cone Characteristics

The mean tree dbh was  $34.6 \pm 1.21$  cm with no significant differences in dbh among the sampled trees at  $p=2.44$  (Fig. 1). Mean cone weight per tree ranged from 9.3 to 42.4 g, giving an overall mean cone weight of  $28.5 \pm 1.20$  g and also showed no significant differences between cones from individual trees ( $p= 2.43$ ). The mean cone width per tree ranged from 2.1 to 3.4 cm with no significant differences ( $p=0.09$ ). Correlation analysis of the three variables dbh, cone width, and cone weight resulted in a greater positive correlation between tree dbh and cone width ( $r = 0.082825$ ) than tree dbh and cone weight ( $r=0.005022$ ). The correlation between cone weight and cone width had the strongest positive relationship  $r= 0.796554$ .

Many studies have shown positive correlations between cone and pine seed production with its DBH and height used as predictors [10,37,38]. Cone and seed production in other pines has also been shown to be positively influenced by larger basal areas [22,39]. Other factors such as stand density, climatic variations, and the morphological parameters of trees also influence their reproductive capacity [40–42]. It was reported that in some pines (for example Aleppo), sub-humid climate enhances cone production over semi-arid conditions. The environmental factors in all the sites of this study were the same and given the homogeneity of the stand, between-trees differences in cone abundance were minimal [43–45].

However, this is not always the case as previous studies on *P. sylvestris* and *P. koraiensis*, clonal seed orchards that were expected to express homogeneity in phenotypic traits, showed variations in cone and seed characters [46–48]. These characters were as follows: cone length, cone width, cone weight, apophysis length, apophysis width, number of filled seeds, the total number of seeds, seed length, seed width, seed weights, opened carpel, and the total number of carpels [49,50]. Seed production from seed orchards can be enhanced by improving cone traits such as cone abundance per tree, number of seeds per cone, and seed weight [43,51].

#### 3.2 Cone Characteristics and Seed yield

For the groups within the first exposure treatment L1, H1, N1, and W1 the number of seeds released per cone ranged from 0-81, 0-128, 1-103, and 4-107 respectively. The seed yield reduced with progression in time with much of the seed released within the first hours (Fig. 2). Overall, there were significant differences ( $p=0.00$ ) within the groups based on extraction period and cone physical characteristics. The current findings corroborate with previous work that, other factors notwithstanding, the size of cones is positively correlated with seed yield. The wide cones released more seeds [32,36,52]. High temperatures cumulatively favor seeds released over time [13,53,54]. Similarly, this study showed a positive effect on seed release cumulatively with an increase in exposure period regardless of the cone’s physical characteristics. The extraction rate was highest within the first six hours and lowest for narrow cones at the end of 24 hours under controlled conditions.

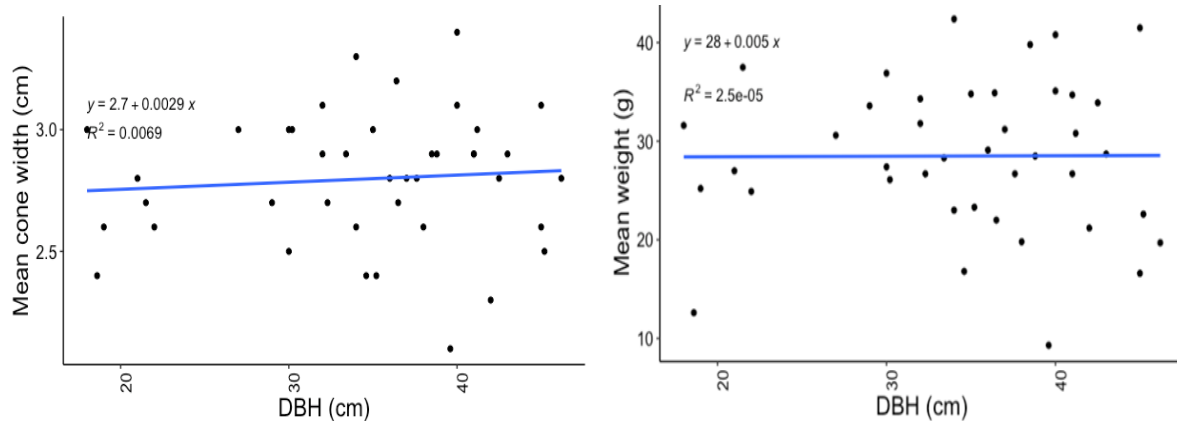


Fig. 1. Relationship between tree dbh and cone characteristics

In all three phases of extraction, cone physical characteristics showed a significant influence on seed yield at  $p=0.05$ . In the first phase of extraction, significant differences in seed yield emerged between light and narrow cones, whereas heavy and wide cones showed no significant differences throughout the extraction period at  $p=0.24$ . F test between groups revealed a significant difference in seed yield as a result of cone width as the other groups showed no statistical differences in seed yield.

The mean cumulative number of seeds per cone from each of the categories was as follows: L=28, H=50, N=38, and W=56 (Fig. 3) Optimum seed release was observed at the end of the first 6 hours with the highest yield from wide cones with 64% of the total seed released. Cone weight and cone width were found to be positively correlated with the number of seeds released whereas an increase in size showed an increase in the number of seeds released.

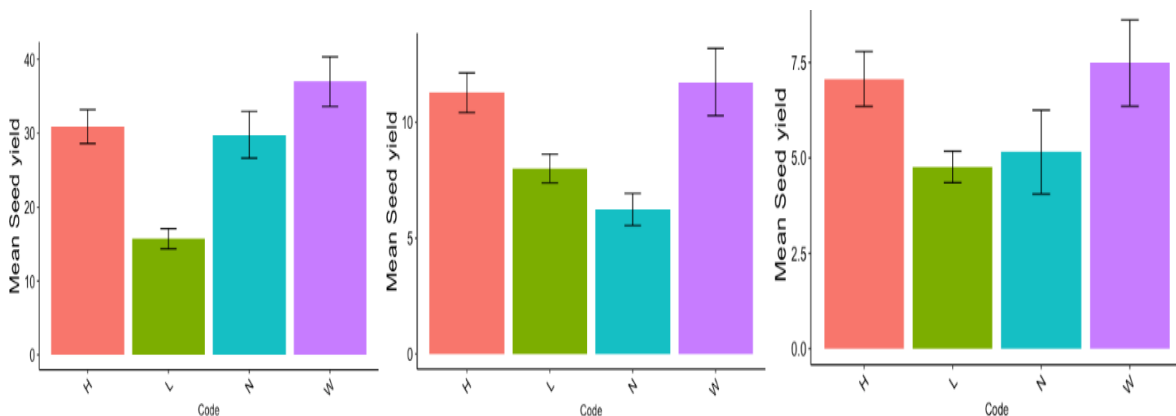
The extraction rate was highest within the first six hours and with wide cones demonstrating the best performance yielding an average of six seeds per cone per hour (Fig. 4). The extraction rate in all the groups dropped as the extraction period increased with the lowest observed in narrow cones 0.4 seeds per cone per hour between 12 and 24 hours.

Combinations of cone physical traits showed that heavy and wide cones performed better in seed release both at 12 and 24 hours (Fig. 5). The slowest cone opening was observed in natural

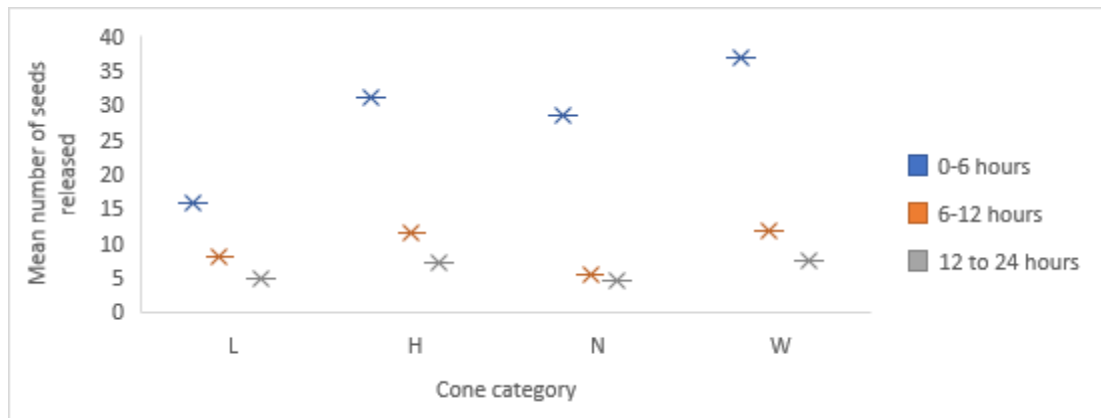
sun-drying conditions while others did not open at all. This was attributed to fluctuating temperatures as some days were not so sunny. Many factors have been attributed to poor cone opening such as high moisture levels, due to early harvesting, fungal and insect damage as well as case hardening of cones during storage [33,37,39,46,55]. In this study, most of the cones that did not open were the narrow ones subjected to natural sun-drying conditions for 21 days. These cones had signs of either physical damage or fungal attack. Delays in *P. patula* and *P. radiata* cones opening due to moisture levels had been ruled out in a previous study that showed temperature had a significantly greater influence on cone opening than moisture content [13,17].

Seed yield from cones exposed to natural seed extraction for up to 21 days in drying beds was observed to have significant differences based on cone characteristics (Fig 6, 7 & 8). Narrow and light cones showed no significant difference in mean cumulative seed at the end of the 21 days ( $p=0.67$ ).

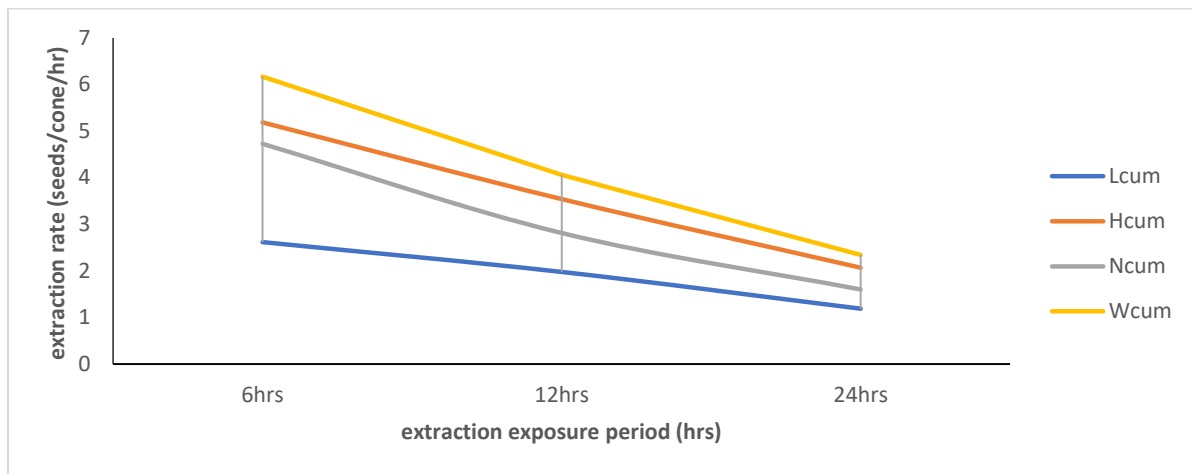
Narrow cones exposed to natural drying for seed extraction were observed to experience more delays in the opening as this category had the highest number of cones, 13 out of 30, that had not opened for seed release by the end of the 21 days. However, the light cones had the overall lowest mean cumulative seed yield. The observed mean number of seeds in each category was LDB=17±0.02 HDB=52±0.04 and NDB=22±0.04 (Fig. 8).



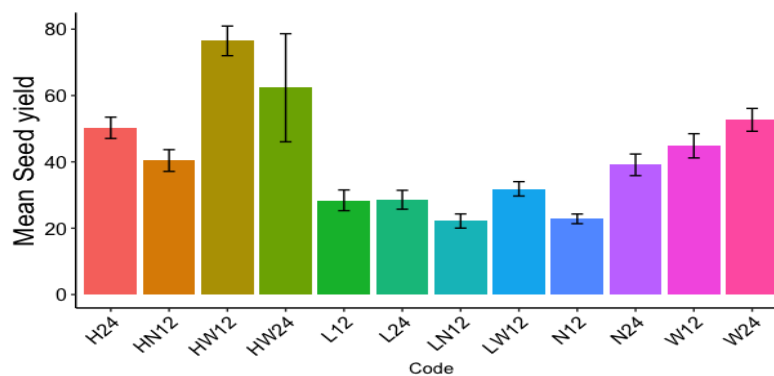
**Fig. 2. Mean seed yield at the end of extraction periods 1, 2 & 3 based on the cone characteristics Heavy (H), Light (L), Narrow (N), and Wide (W)**



**Fig. 3. Seed release concerning extraction exposure periods and cone characteristics**



**Fig. 4. Extraction rates of seeds from Light (L), Heavy (H), Narrow (N), and Wide (W) cones**



**Fig. 5. Comparison seed yield extracted under combined factors; extraction exposure period and one or two of the cone characteristics**

Where H24=heavy cones exposed to 24 hours of extraction, HN12=heavy yet narrow cones exposed to 12 hours of extraction, HW12=heavy and wide cones exposed to 12 hours of extraction, HW24=heavy and wide cones exposed to 24 hours of extraction, L12=light cones exposed to 12 hours of extraction, L24=light cones exposed to 24 hours of extraction, LN12=light and narrow cones exposed to 12 hours of extraction, LW12=light yet wide cones exposed to 12 hours of extraction, N12=narrow cones exposed to 12 hours of extraction, N24=narrow cones exposed to 24 hours of extraction, W12=wide cones exposed to 12 hours of extraction, and W24=wide cones exposed to 24 hours of extraction

**Table 1. Variation in seed yield based on cone characteristics and phases of extraction**

<b>Treatment</b>	<b>L1</b>	<b>H1</b>	<b>N1</b>	<b>W1</b>	<b>L2</b>	<b>H2</b>	<b>N2</b>	<b>W2</b>	<b>L3</b>	<b>H3</b>	<b>N3</b>	<b>W3</b>
Mean seed yield ( $\bar{x} \pm se$ )	15.7±1.38	30.9±2.31	29.8±3.15	37.0±3.36	8.0±0.62	11.3±0.86	6.2±0.69	11.8±1.45	4.76±0.42	7.1±0.72	5.2±1.10	7.5±1.13
SD	15.49	27.58	25.59	26.05	6.96	10.28	5.61	11.25	4.66	8.61	8.93	8.74
CI	2.733	4.561	6.292	6.731	1.227	1.699	1.379	2.907	0.821	1.424	2.194	2.258
% seed yield	55	63	74	66	28	23	14	21	17	14	12	13
% cumulative seed yield	55	63	74	66	83	86	88	87	100	100	100	100
Performance-based on the extraction phase	64				22				14			

\*L,H,N,W : cone characteristics light, heavy, narrow, wide and wide cones, 1,2,3: seed extraction exposure periods from 0 to the 6<sup>th</sup> hour, 6<sup>th</sup> hour to the 12<sup>th</sup> hour, and the 12<sup>th</sup> hour to the 24<sup>th</sup> hour,  $\bar{x}$ : group mean, SE: Standard Error, SD: Standard Deviation, CI: Confidence Interval

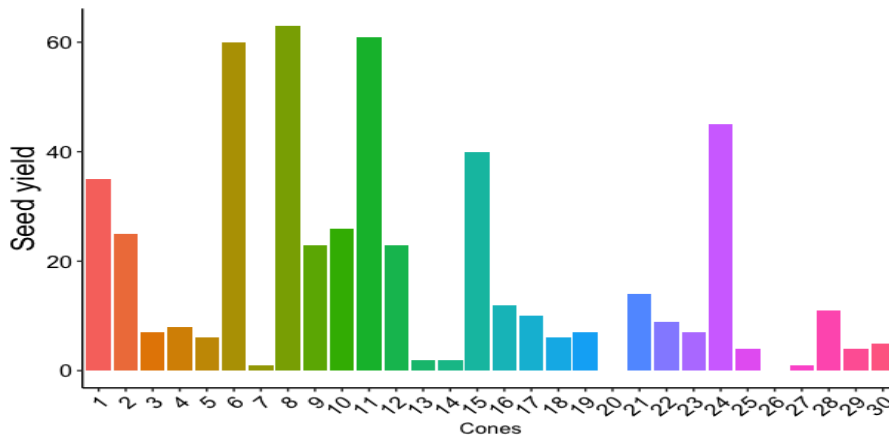


Fig. 6. Cumulative seed yield of individual light cones exposed to natural drying in seed extraction beds up to 21 days

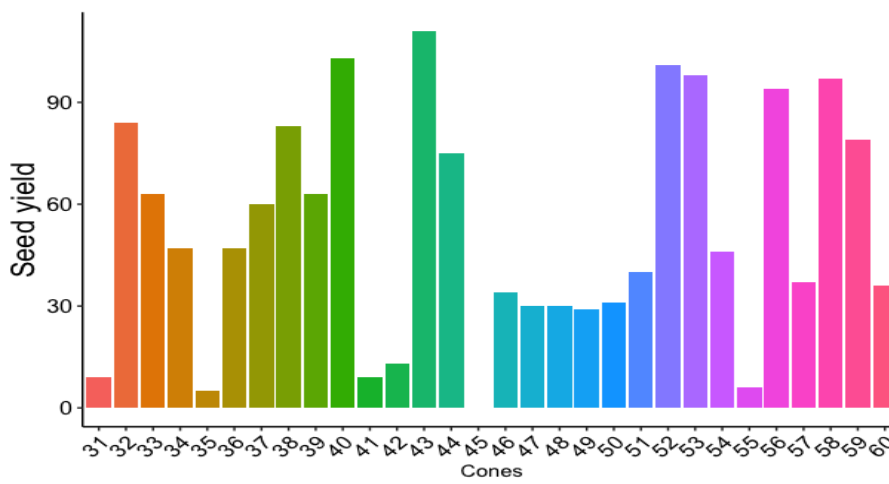


Fig. 7. Cumulative seed yield of heavy cones exposed to natural drying in seed extraction beds up to 21 days

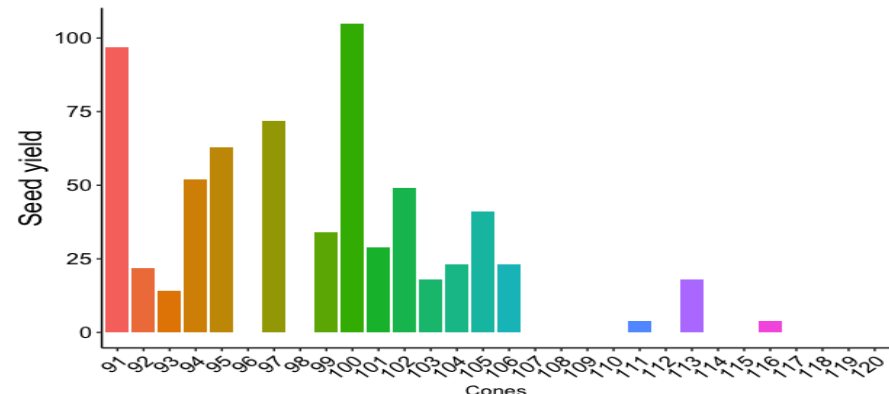
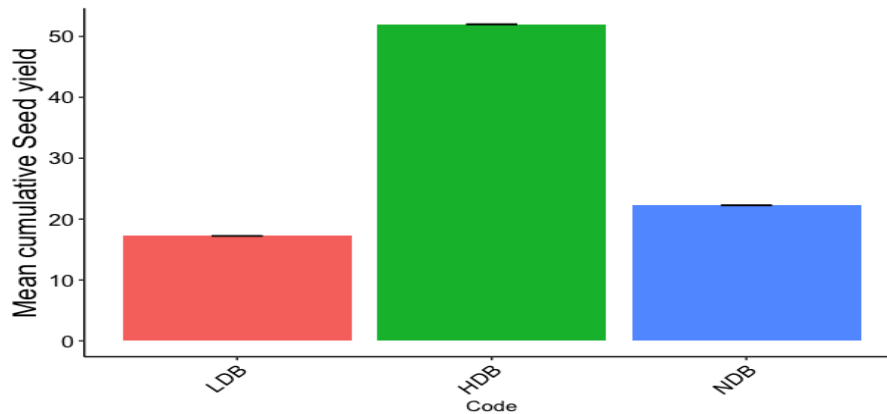


Fig. 8. Cumulative seed yield of individual narrow cones exposed to natural seed extraction for up to 21 days





**Fig. 9. Mean cumulative seed yield of light (LDB), heavy (HDB), and narrow (NDB) cones exposed to natural seed extraction in drying beds**

#### 4. CONCLUSIONS

In this study, there is a positive correlation between cone width, cone weight, and tree DBH with any increase in diameter having a positive effect on the cone size and weight. This study showed that for *P. patula*, cone width had a greater influence on the amount of seed release than cone weight. When considering efficiency in mechanized seed extraction, the first six hours were shown to be optimum for seed release. Thus, cone sorting for wider cones for extraction at 65°C for at least six hours would yield a greater part of the seeds in artificially heated timed kilns.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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