EFFECT OF PATULA PINE (*Pinus patula*) CONE PHYSICAL CHARACTERISTICS AND EXTRACTION PERIOD ON SEED YIELD AND GERMINATION IN LONDIANI, KENYA

ONYANGO ALICE ADONGO

A Thesis Submitted to the Graduate School in Partial Fulfilment of the Requirements for the Master of Science Degree in Natural Resources Management of Egerton University

EGERTON UNIVERSITY

APRIL 2024

DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented in this university or any other for the award of a degree.

Onyango A. Adongo

Signature: Date:

NM11/13720/19

Recommendation

This thesis has been submitted with our approval as University supervisors

Dr. Shadrack Kinyua Inoti (PhD)

Signature: Date:

Department of Natural Resources Management

Dr. Maara T. Nelson (PhD)

Signature: Date:

Department of Natural Resources Management

COPYRIGHT

DEDICATION

This thesis is dedicated to my children namely; Bradley and Ashley.

ACKNOWLEDGEMENTS

My sincere gratitude goes to God our creator for enabling me in all stages of this work. Kenya Forestry Research Institute management through the KEFRI graduate school programme and staff at the Kenya Forest Seed Center are acknowledged for the support accorded during the entire process of this work. I hereby acknowledge lecturers at the Department of Natural Resources Management under the leadership of Dr. Margaret Githiri for the support accorded since the inception of this work.

The entire thought process, shaping of approaches and implementation of this study would not be any better if it were not for the input, guidance and support of my supervisors Dr. Shadrack K. Inoti and Dr. Nelson Maara. I thank Dr. J. M. Kimondo for his valuable suggestions and helpful comments during the thesis review phase.

I would like to acknowledge Mr. Joram M. Mbinga, a tree improvement expert whose work provided for the establishment and maintenance of the seed orchard from which, materials used in this study were sourced.

My gratitude is further extended to foresters; Richard Siko, and Anne Ndigirigi, technologists; Shadrack Omambia, Lidia Khibali, Denis Olemayan, tree climbers; David Ndirangu and Fredrick Kirwa, and driver; Mr. Samson Tobit for the assistance accorded in fieldwork phase of this study.

I appreciate my young family, Bradley and Ashley for cooperating with me when need arose to work from home. Many thanks to my parents; Mr. Z. K Onyango and Mrs. T. K. Onyango for their spiritual and moral support in the entire course of my studies.

ABSTRACT

Pinus patula in Kenya is an exotic plantation tree species grown for commercial production of sawn wood and pulpwood. Propagation is by seed and grafted seedlings or cuttings for clonal seed orchards. The conventional way of *P. patula* seed extraction is by drying in beds in direct sunlight. This study was set up to investigate the interactions of cone physical characteristics, exposure duration to artificial seed extraction at a fixed extraction temperature and the germination temperature on seed release and subsequent seed germination. The hypothesis tested was that there was no relationship between cone characteristics, extraction exposure duration, seed yield and seed germination performance. Forty-five trees for cone collection were systematically selected with a random start from Kamara block, Londiani forest. 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. Based on the variations in physical attributes and extraction exposure periods, seed extraction potential of cones and germination responses of seeds to thermal treatments was determined. The lowest mean number of seeds released observed was 28, from light cones, while the highest mean was 56 from wide cones. This study showed that for *P. patula,* cone width had a greater significant (p=0.001) influence on the amount of seed release than cone weight. There were significant differences (p=001) 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°C demonstrated the highest germination percent at 90% while the lowest was 20% from light cones exposed to similar germination temperature conditions. 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. Based on this study, foresters and germplasm producers can improve protocols for scoring of seed sources based on cone characteristics to infer germination potential of *P. patula*.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF PLATES

LIST OF TABLES

LIST OF ABBREVIATIONS AND ACRONYMS

℃ – degrees Celsius

cm - centimetres

dbh – diameter at breast height

g – gram

g/l – grams per litre

GA - Gibberellic Acid

hrs - hours

ISTA – International Seed Testing Association

j/g – joules per gram

KEFRI – Kenya Forestry Research Institute

KFS – Kenya Forest Service

m- meters

m.a.s.l – meters above sea level

smc – seed moisture content

wt – weight

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Pines have been introduced to regions outside their native climate niche, particularly to regions with cooler, wetter and less seasonal climates (Essl *et al*., 2011; Sastry *et al*., 2019). Pines are one of the fast growing exotic trees species established for commercial plantation development in East and Southern Africa (Owino & Ndinga, 2004; Pinkard & Keenan, 2012). In Africa, *Pinus* species, mostly originating from the American or Asian tropics and sub-tropics that are widely cultivated and commonly planted species include *P. caribaea*, *P. elliottii*, *P. greggii*, *P. kesiya*, *P. maximinoi*, *P. patula*, *P. oocarpa*, and *P. tecunumanii* (Marunda *et al*., 2019). *Pinus patula* is a widely planted species in the world with a geographic range from Ecuador to a latitude 31° south in Africa and extending to 42° southern latitude in New Zealand.

Patula pine (*Pinus patula*) is a straight bole tree with its origin from Mexico (Perry, 1992). In Mexico, this species is used for: pulp, sawn timber, veneer and plywood, boxes, pit props, sleepers, telegraph poles and construction wood and, ecologically for protection of watershed areas as well as for conservation and restoration of degraded soils. It is has a wide distribution range including southern and East Africa where it is commercially planted for sawmill timber, pulp and floors (Dvorak *et al*., 2012; Essl *et al*., 2011).

The species is mainly planted in African countries but is also used in Australia and Asia (Australia, New Zealand, Papua New Guinea, and India) and on a smaller scale South America (Argentina, Brazil, Colombia). The total area planted until 1970 was approximately 450,000 ha. The countries planting the largest areas in this period were South Africa followed by Swaziland, Zimbabwe, Kenya, and Madagascar (Nöjd & Isango, 2003) . In Kenya, *P. patula* represents 27% of plantation species among others such as *Cupressus lusitanica* and Eucalyptus species (Kuria *et al*., 2019). It has been grown in plantation scale for industrial production for pulpwood and sawn wood hence exerting a high demand for seed extraction.

Sustainable plantation forest production demands a continuous supply of high-quality seeds for the production of seedlings in the nursery or for direct sowing. One of the species commercially planted is the pines (Aniszewska *et al*., 2020; Barbour, 2007; Savill, 2019). The shortages of tree seeds are happening against a background of high deforestation and low rates of tree planting and projected deficits in timber supply (Marunda *et al*., 2019). This, coupled with a growing backlog in tree planting, a limited plantation forest resource, increasing temporarily unplanted areas, potential negative impacts of climate change, and threats of diseases and pests, there is an urgent need for regions worldwide to address sustainable germplasm production issues (MacDicken *et al*., 2015; MacDicken, 2015; Miura *et al*., 2015).

Seed extraction from pines globally is challenging to the forestry sector (Bhat *et al.*, 2017; Reyes & Casal, 2001) with most countries using the conventional methods for seed extraction from cones which entails drying in beds to facilitate seed release. This is a weather dependent process which is particularly slower and less efficient in moist, cool temperate climates since an increase in the atmospheric humidity may cause a reclosing of the cones (Willan, 1984). However, in spite of this, it is the most economical, convenient and effective method of seed extraction for many cone bearing species as the process in most countries relies on natural heat energy from the sun for drying cones in canvas or drying beds.

Previous studies have reported that pine cones open to release seed through a combination of temperature and humidity (Aniszewska, 2013; Reyes & Casal, 2001) which varies widely in the pine growing regions of the world. There is scanty literature on *P. patula* seed extraction efficiency with most studies focusing on other pine species (Ayari & Khouja, 2014; Bilir *et al*., 2008; Reyes & Casal, 2001; Singh *et al*., 2017; Wyse *et al*., 2019). Earlier work has also revealed that the primary factor for seed release is the degree of opening (Calvo & Nunez, 2000). The degree of opening influences scale deflection for seed release in pines which is mostly affected by the underlying conditions; temperature and humidity (Aniszewska & Zychowicz, 2020; Harlow *et al*., 2019).

In Kenya, *P. patula* (Plate 1) is the sole pine species used for commercial plantations establishment following the withdrawal of *P. radiata* as a plantation species due to its susceptibility to *Dothistroma pinii* fungal disease. In addition, improvement strategies such as establishment of clonal seed orchard for production of quality germplasm has over the years been undertaken with selection of superior mother trees based on bole characteristics (Joshi *et al*., 2016; Kokutse *et al*., 2016; Mbinga *et al*., 2022). Techniques have been developed for rapid extraction of seed through soaking and oven drying for other species and have shown no effect on seed quality and germinability (Bhat *et al*., 2017). Seed processing and handling can contribute to the overall seed losses by decrease in viability (Costa *et al*., 2016). Artificial drying encourages seed release and scale opening but the adverse effect of other underlying factors such as cone characteristics and extraction exposure periods, on seed quality and germinability is yet to be established for *P. patula* in Kenya. The lack of understanding of these effects has led to a combined reduction of available seed which is in high demand for pine plantation establishment especially in the highlands, hence the contribution of the study.

Plate 1: Photograph of *P. patula* tree (foreground) and plantation (background- Left) at Londiani, Kenya.
Source: Photograph by Onyango A.A, 24/10/2021.

1.2. Statement of the problem

In Kenya, *P. patula* is grown as a commercial plantation species, hence, a high demand for a sustainable supply of quality seeds for direct propagation or raising of seedlings. The common practice of *P. patula* seed extraction in Kenya, entails drying of cones in drying beds for fourteen days under the direct sun. Cone collection is a function of season, hence seed extraction is limited to specific times of the year. With shorter periods of cone collection and longer periods of seed extraction, there is high chance of compromising seed germination. The prolonged period also makes the process uneconomical. The narrow periods of harvesting of pine cones are also dependent on season. *Pinus patula* and *Cupressus lusitanica* are coniferous species grown in Kenyan plantations for commercial uses. In coniferous species, the efficiency of cone opening relies on the size and shape of the cones as more scale deflection happens to cones with circular structure such as *C. lusitanica* than the tapered ones like *P. patula* whose next to stem part does not open. This explains the advantage *C. lusitanica* in releasing high numbers of seeds and in a shorter period than *P. patula*. Thus, for *P. patula* there is need to reduce the extraction time and improve extraction yield in terms of quantity and germination performance using artificial heat sources, hence the need for this study.

1.3 Objectives

1.3.1 Broad objective

To determine the effect of *Pinus patula* cone physical characteristics, exposure period and germination temperature on seed release and viability for improved germination performance towards achieving sustainable plantation forest development.

1.3.2 Specific objectives

- **i.** To determine the effect of cone width and exposure period on seed release.
- **ii.** To determine the effect of cone weight and exposure period on seed release.
- **iii.** To analyze the correlations between cone width, exposure periods and germination temperature on viability.
- **iv.** To analyze the correlations between cone weight, exposure periods and germination temperature on viability.

1.4. Hypotheses

- **i.** H_0: *Pinus patula* cone width and exposure period have no influence on seed release.
- **ii.** H_0: *Pinus patula* cone weight and exposure period have no influence on seed release.
- **iii.** H_0: There is no correlation between cone width, exposure periods, germination temperature and seed viability.
- **iv.** H_0: There is no correlation between cone weight, exposure periods, germination temperature and seed viability.

1.5. Justification of the study

While seed production data are readily available for agricultural crops, such information is generally not available for forest tree seeds. *Pinus patula* is a priority commercial tree species in Kenya, and therefore requires availability of data on seed extraction efficiency and germinability. In pine growing regions, many studies have been conducted to determine the threshold temperatures for safe seed release in fire prone areas where heat in the form of fire facilitates natural regeneration of pine. In the case of pine growing in Kenya, cone characteristic based responses to extended extraction periods for optimum seed release and safe germination is yet to be documented. This study sought to improve on the extraction practice by use of empirical data as a demonstration of the efficiency of artificial seed extraction technique that will shorten the extraction time, enhance seed release, and have less or no adverse effect on seed yield and germinability parameters of the species. This will further enhance the planning processes for seed collection and production especially for germplasm producers. The findings of this study are expected to improve on the existing knowledge and procedures of seed extraction from cones by providing new insights into the cone selection process which will be beneficial to germplasm users and producers.

1.6. Scope /Limitations/Assumptions

1.6.1. Scope

The cones for seed extraction were collected from a seed orchard in Kamara block of Londiani forest. Seed extraction and germination were conducted under laboratory-controlled conditions at Kenya Forestry Research Institute, Londiani. This study correlated the cone properties (width and weight), extraction exposure periods and germination temperature to seed release and seed germination. Germination was the indicator for the assessment of the effect of temperature on the embryo of freshly extracted seed.

1.6.2. Limitations

Cone and seed production have been attributed to environmental, phenotypic and genetic similarities and variations. Reduction in cone and seed production due to climatic variations could have potentially affected the availability of cones and seed for this study. There could be variations in germination that the study could not prove that the better seed producers and performers were from the same mother tree or were otherwise genetically different. The experimental design used in this study, factorial completely randomised design, was expected to greatly minimise these limitations.

1.6.3. Assumptions

It was assumed that environmental and genotypic influences had been greatly reduced since the source of cones was a clonal orchard that had been established in a favourable ecological region (within the right climatic range and site conditions) for growth and the mother trees were superior performing selected trees.

1.7 Definition of Terms

Cone – reproduction part that produces seeds in pines

Conifer - a tree that bears either soft or woody cones

Cone physical characteristics – cone width and cone weight

Extraction period - time taken for cone to release seed

Extraction rate – speed at which a specific number of seeds are released within a given period

Exposure – contact with conditions of the surrounding

Resin – plant exudate that seals scales in cones

Seed cone – female reproductive part

Seed germination - the process by which plant growth is initiated from a seed into a seedling

Seed orchard - plantation established from cuttings of trees or seedlings of known identity established for seed production

Seed quality –physiological soundness and health status of seed

Serotinous trees - trees whose cones remain closed at maturity only to open in response to high temperatures

CHAPTER TWO
LITERATURE REVIEW

2.1 Introduction

In this chapter, previous findings in relation to the topic under study will be reviewed. This includes the reproduction, distribution, use and propagation of pines. Variations in cone physical properties and their influence on seed yield will be highlighted. This chapter also touches on the advances made in the seed extraction process of coniferous species through artificial manipulation of temperature. The chapter also touches on techniques suggested to minimize viable seed losses and understanding the seed germination behaviour in relation to moisture content. Theoretical and conceptual frameworks will be discussed as well as the research gaps.

2.2 Reproductive biology of conifers

a) ***Cone development*** *:* Conifer families are broadly classified into *Araucariaceae*, *Cupressaceae*, *Pinaceae*, *Podocarpaceae*, *Sciadopityaceae*, and *Taxaceae* (Yang *et al.,* 2022). The reproductive structures in conifers are separate: either on the same (monoecy) or different plants (dioecy). In monoecious species, pollen and ovulate strobili are often spatially separated. Pines are monoecious with pollen strobilli (male) and seed (female) cones borne separately on the same tree. Pollen is produced on lower branches and seed-bearing cones on upper branches. In most species, these two reproductive parts occur on separate shoots, yet in some species they are on the same shoot. The spatial separation of reproductive structures is one of the mechanisms in pines that reduce self-fertilization by increasing the chance for seed cones to receive pollen from other trees through wind pollination (Neale & Wheeler, 2019).

b) ***Fertilization and seed development:*** The duration required for pines to reach the stage of formation of pollen and seed cones varies considerably among species. This ranges from less than 10 years (*P. attenuata*, *P. clausa*, *P. contorta*, *P. greggii*, *P. pungens* and *P. rigida*) to over 30 years (*P. flexilis*, *P. edulis*, *P. resinosa* and *P. lambertiana*). Species in high fire frequency environments most of the time begin their reproduction earlier than others (Fernando, 2014). Timing of pollen shed and seed cone receptivity in pines vary but are often offset, with pollen shed typically occurring first (by one to several days). Temperature and humidity have been shown to influence the pollen maturation and pollen shedding (Nelson *et al.,* 2012). For optimal pollination, peak pollen release should coincide with peak seed-cone receptivity. Dichogamy occurs when pollen release and receptivity do not coincide. Protandry occurs when peak pollen release precedes peak seed-cone receptivity. Protogyny occurs when peak seed-cone receptivity precedes peak pollen release. While environmental factors are the major causes of the asynchrony between pollen release and seed cone receptivity, irregular cone production has been considered an adaptive trait in harsh growing conditions or periods. It is an assumption that irregular cone production is an effective trade-off strategy for resource use and allocation. In periods of cone losses, the more resources can be saved for growth and maximised reproduction for the abundant years (Goroshkevich *et al.,* 2021; Losada *et al.,* 2019; Redmond *et al.*, 2019).

In lodgepole (*Pinus contorta*) and loblolly pines (*Pinus taeda*), unpollinated ovules do not continue development and if their number is greater than 80 %, the entire seed cone will abort and eventually falls off. This mechanism minimises wastage of food resources in sustaining seed cones with lower potential of reproducing future progenies (Bonner, 1987). Some pines exhibit masting behaviour. The synchronous production of large seed crop and at long intervals by a population of plants is known as masting. Inter-event times of seed (cone) production of longleaf pine (*Pinus palustris*) has been quantitively studied in efforts to understand forest dynamics and develop sustainable management strategy. In this analysis temporal cone and seed production behaviour was used to characterize longleaf pines seed and cones. It was concluded that there exist positive relationships between seed (cone) production and inter-event time for longleaf pine (Chen *et al.*, 2021).

2.3 Importance of pines

Conifer plantations form the backbone of forestry in many regions of the world (Bravo-Navas & Sánchez-Romero, 2022; Pinkard & Keenan, 2012). Hence plantation areas are large and have rapidly expanded since the beginning of the 20th century (Muhtaman *et al.*, 2000; Richardson *et al.*, 1994). In all continents, and for the vast majority of regions, numbers of naturalized Pinaceae are higher than those for Cupressaceae. Studies have shown that, across the globe, the successful adaptation of conifers is co-determined by factors such as climate match, habitat diversity, biogeographic features and introduction effort. These findings underpinned the role of climatic suitability as a selective filter for successful adaptation (Essl *et al*., 2011).

Pine forests are the dominant vegetation in many parts of North America, Europe and Asia (Bialobok & Zelawski, 1976; Payn *et al*., 2015). Pines exhibit a wide range of ecological strategies; some species fill early successional roles whereas others are late-successional trees (Barbour, 2007; Martínez-Ramos *et al*., 2016). 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 (Ramírez-Mosqueda *et al*., 2018; Richardson *et al*., 1994).

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 (Agne *et al*., 2022; Haymes & Fox, 2012). 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.

Most authors agree that there is a relationship between the production of cones and seeds and various tree size measurements, such as total height (Moya *et al*., 2007), trunk diameter (Ayari *et al*., 2012; Bravo *et al*., 2017; Ganatsas *et al*., 2008; Moya *et al*., 2009; Sutton *et al*., 2002) or both parameters (Moya *et al*., 2008), in addition to the tree crown size (Ayari *et al*., 2011; Iwaizumi *et al*., 2008). Other studies have linked, on one hand, tree crown variables and cone number, and on the other hand, cone number per tree and seed mass per tree (Ayari & Khouja, 2014; Ayari *et al*., 2012). Yet another study on *P. palustris* ruled out the use of solely tree size as a weak predictor of cone production because according to this study site conditions and interactions with neighbouring trees were factors that also contributed to reproductive heterogeneity, and thus had to be considered when relating cone production to size (Haymes & Fox, 2012).

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 (Keenan *et al*., 2015; Köhl *et al*., 2015). *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 (Adegbehin, 2016; Crous *et al*, 2009). The species was introduced in South Africa from Mexico in 1907 and first raised in plantations in the Natal Midlands at Cedara in 1914 (Nöjd & Isango, 2003; Saenz-romero & Vargas-Hernandez, 1994). It is likely that plantations of this species later raised in Eastern African countries had their seed source from South Africa. The area of *P. patula* plantations as percentage of softwood established up to 1970, was approximately 79% for Malawi, 41% for Tanzania, 37% for Uganda and 33% for Kenya. In these countries the species is a major source for saw logs, pulp and paper (Adegbehin, 1982) .

Pinus patula is a straight bole monoecious plant that grows to a height of 30 m or more and attains a diameter at breast height (dbh) of up to 1.2 m. It grows in altitude of 1,000-3,000 m.a.s.l with a mean annual temperature of 10-25°C and a mean annual rainfall of 1,000-2,000 mm. The common soil features include acidity and good moisture supply. 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 (Brister & Fry, 1966; Kuria *et al*., 2019; Ngugi *et al*., 2000). 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 (Orwa *et al.,* 2009).

2.4 Pine seed production and propagation

Propagation of *P. patula* is mainly by seed. *P. patula* is a serotinous pine and thus produces serotinous cones (Orwa *et al*., 2009). Serotinous cones retain their seeds for one or more years following maturity (Lamont *et al*., 2020; Tapias *et al*., 2001), with seed release and maximum dispersal often occurring in response to an environmental stimulus such as high temperature (Romero & Ganteaume, 2020; Wyse *et al*., 2019). Examples of other closed cone pines that can keep the seed in cone for several seasons only to open after a fire and thus ensure regeneration include *Pinus halepensis*, *Pinus radiata*, *Pinus banksiana*, and *Pinus brutia* (Calvo & Nunez, 2000). The hygroscopic nature of woody cones explains the dehiscence behaviour which increases with decrease in atmospheric humidity (Aniszewska, 2013; Bae & Kim, 2020; Quan *et al*., 2021).

Gradual opening of scales is a favourable adaptive feature for the species. This feature allows for the availability of seed for germination, regeneration and establishment at a time when the environment will give the best chance for survival (Dvorak *et al*., 2001; Sáenz-Romero *et al*., 2011; Saenz-romero & Vargas-Hernandez, 1994; van Zonneveld *et al*., 2009). The gradual release of seeds, available from different periods is a strategy that highly encourages survival and expansion of the species in natural regeneration conditions (Duryea, 1987). Another advantage associated with gradual opening of cones is the increased chances of escaping predators (Samano & Tomback, 2003; Tíscar, 2019). Large quantities of seed are released in the dry period of the year, when there are high possibilities for contact with the soil. The average number of seeds per cone of *P. patula* is approximately 125 in its natural habitat and there are about 118,000 seeds per kilogram of seed. Expected recovery performance ranges of between 64,000 (54%) and 96,000 (81%) seedlings per kilogram. Seeds from *P. patula* seed orchards demonstrate better performance at 70-85 % germination in the nursery conditions (Nel, 2002).

Vegetative propagation by grafting or air-layering is possible, as clonal orchards have been established using these methods (Bilir *et al*., 2008; Sivacioğlu & Ayan, 2008; Weng *et al*., 2020). The use of cuttings for clonal propagation is limited by rapid initiation of ontogenetic aging in the plants, typified by the early onset of reproductive maturity and resulting in variation in rooting, growth habit, flowering and leaf morphology (PROTA, 2016) .

2.5 Pine seed extraction

Seed extraction from the cones of numerous forest tree species has been considered as a complicated procedure decided by taxonomic characteristics. Cone properties such structure, size, anatomy and shape have been shown to influence the choice of method for obtaining seed and the quantities of seed released. Factors such as temperature, relative humidity and moisture content has been shown to affect the opening of pine cones (Harlow *et al*., 1964; Horstmann *et al*., 2022; Keeley, 2012). Cell properties (moisture, shape and size) of the scales also determine the reflexes and scale deflection required for seed release. Seed extraction process involves the contraction of cell walls into the space previously occupied by water and a decrease in the volume of the cones content. Changes in levels of hydration during drying causes changes in the shape of cones' scales and often leads to reclosing of cones (Quan *et al*., 2021; Tulska *et al*., 2021).

Most development of techniques to address the difficulty have an incline to research pine seed extraction because of the reality that the seeds of that species are in greatest demand (Li *et al*., 2021; Robb, 2020). Due to pine cone sensitivity to changes in humidity and temperature during opening for seed release, alternate methods to natural sun drying such as kiln drying have been developed in most moist and cool countries. Kilning is described as the drying of cones in a controlled, warm, dry environment to flex the cone scales and allow seeds to be extracted. Artificial heating provides for control of moisture and temperature thereby shortening the period of seed extraction (Ghildiyal *et al*., 2008).

Among other factors considered when designing artificial seed extraction devices is simplicity of operation, minimal space required, flexibility to increase or reduce drying capability, and minimal investment in system. Kilns are designed to remove moisture and induce flexing in conifers by use of essential elements: (i) heat for evaporating moisture, (ii) air circulation to conduct heat, (iii) control of temperature and humidity to prevent injury to the seeds and (iv) a tray or shelf system to expose cones to the air current. Cone pretreatment before artificial heating for seed extraction has showed varied to no responses in cone opening in a number of species in the Pinus genera (Singh *et al*., 2017).

In Kenya, cones are sun-dried in open-sided sheds covered with plastic roofs which open after 2–14 days for seeds extraction. Duration of cone opening takes longer due to the lower temperatures in pine seed extraction regions. On the other hand, germination starts 7–10 days after sowing (PROTA, 2016).

Basically, seeds have been the primary source of propagules especially in making of hybrids for enhanced productivity of specified features or product from the pine crosses (Kanzler *et al*., 2014; Sastry *et al*., 2019). In East Africa natural regeneration is often after a fire event. Seeds germinate abundantly after a fire (PROTA, 2016).

2.6 Influence of pine cone physical characteristics on seed yield

Conifers have displayed between and within species cone differences in terms of cone morphology, density, mass, weight, colour, degrees of asymmetry, differences in scale tension, effect of environment on bonding strength and genetic differences in bonding agent (Angaine *et al*., 2021, 2020; Ayari *et al*., 2011; Iwaizumi *et al*., 2008; Perry & Lotan, 1977). Observation of morphological characteristics of cones and seeds has been suggested to guide selection of seeds from seed stands, establishment of seed orchard, development of seedling nurseries, reforestation and forestation in degraded forests (Ribeiro *et al*., 2022; Tomback *et al.,* 2022). Strong relationships have been observed between cone and seed morphological characteristics and seed quality whereby, for instance, cones sizes influenced the number of seeds per cone (Kaliniewicz & Tylek, 2019; Neyko *et al*., 2020).

The age of mother tree was established to have no influence on cone characteristics (cone and seed weight, number of seeds per cone) and germination capacity of *P. nigra* (Alejano *et al*., 2019), while on the contrary, tree age effects were established for *P. pinaster*, *P. pinea* and *P. echinata* (Callejas-Díaz *et al*., 2022; Ganatsas *et al.,* 2008; Grayson *et al*., 2002) where relationships were established between maternal age, senescence, germination timing and subsequent survival. In other studies, seed colour has been shown to influence germination energy and germination capacity. Darker shades seeds have shown greater rate compared to light-coloured seeds (Bacherikov *et al*., 2022; Udval & Batkhuu, 2013). Additionally, artificial controlled pollination based on morphological characteristics, cone traits, and seed characteristics has demonstrated improved cone and seed production capacity quantified as yield of developed seed per cone for *Pinus densiflora* (Lee *et al*., 2021). Other studies involving characterization of *P. pinea* (stone pine) considered attributes such as cone size, cone survival (following pest and disease attack), and seed output (seed-yield quantity and quality) as pointers in determining influences of environmental and genetic variations on cone productivity (Mutke *et al*., 2005).

The crucial role of serotinous cones to produce a prolific release of seeds, enables a successful re-colonization of burned areas, hence seeds heat tolerance and response to thermal shock is an added advantage for pine seed resources (Lovreglio *et al*., 2007). 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 (Aniszewska, 2010).

The mechanism of scales opening in Pinus genera have also been proved to experience significant differences longitudinally (lengthwise), than cross-sectionally through shrinkage from wet to dry (Harlow *et al.*, 1964). Growth conditions in forest stands can be modified to enhance tree production and development. To achieve this, techniques such as tree release have been used. Tree release is a silvicultural treatment that liberates tree seedlings or saplings by removing the older over topping trees to encourage performance of the young trees. This technique reduces competition and suppression to favour development of individual trees in the understory (Miller *et al.*, 2007). Whereas cone length, diameter, and volume do not vary significantly by stand density, tree dbh, or crown position, cone dry weight differ significantly between released and unreleased trees of shortleaf pines.

Furthermore, released shortleaf pine trees were proved to be more productive as they produced on average 9 more sound seeds per cone than unreleased trees. As previously observed, the number of potentially productive scales per cone varies significantly by placement within the crown with greater numbers found in the upper crown (Grayson *et al.*, 2002). Cone orientation, based on uprightness in *P. strobiformis* has also been reported to have no significant effects on seed extraction rates calculated as number of seeds released per second but there existed cone opening differences over different times of the year (Samano & Tomback, 2003). Information on cone and seed analysis have been suggested as effective guides to the breeders when evaluating seed production from seed orchards (Bramlett, 1977; Karrfalt & Belcher, 1976). Anatomical and morphological characteristics of *P. clausa* (stone pine), *P. echinata* (shortleaf pine), *P. elliottii* (slash pine), *P. glabra* (spruce pine), *P. serotina* (pond pine), *P. taeda* (loblolly pine), *P. mugo* (mountain pine), and *P. palustris* (longleaf pine) cones have been used as clues in the process of identification for biodiversity preservation among the pines (Monteleone *et al.*, 2006; Proctor & Martha, 2016).

Cone size (length and width) and seed size (length, width and weight) have been useful in intra-specific taxonomic distinction between populations of *Pinus greggii* (García-De La Cruz *et al.*, 2015; Fernando, 2014), while parameters like seed weight and length have been used to classify provenances into high and lowland groups in *Pinus caribaea* (Rawat & Bakshi, 2011). Seed source variation with respect to cone, seed and seedling characteristics is well documented for a number of tree species (Tomback *et al.*, 2022; Weng *et al.*, 2020; Hauke-

Kowalska *et al*., 2019). Cone and seed characteristics have been shown to vary among species, provenance and genotypes in pines and are well documented (Udval & Batkhuu, 2013).

Variation in cone size, seed number per cone, seed morphology and seed germination characteristics in relation to stand conditions was analysed for *Pinus sylvestris* L., grown in Mongolia. This study on P. sylvestris showed significant geographic variations in cone and seed morphological traits, and seed germination characteristics. Cone and seed morphological traits were significantly differed within provinces and populations in all measured variables. The criteria used was recommended to guide the selection of best performing and high seed quality seed sources for better productivity and vigorous seedlings (Batkhuu *et al*., 2020). Other studies on extractive uses have characterised pine seeds on basis of morphology, chemical composition, quantity of extracts, nutritional composition (Havelt *et al*., 2020; Lixia *et al*., 2018; Shukla & Kaur, 2018; Valera-Burgos *et al*., 2012). Environmental temperatures heavily weigh on these morphological and chemical quantities as suggested by authors of *P. cembroides* nut extracts study (Reyna-González *et al*., 2019).

Seed orchard plantations have contributed significantly to genetic diversity of pines and to an extent aided the process of natural selection. Genetic variability of pines grown in plantation set up has been shown to differ significantly showing higher values of heterozygosity than of those in natural populations (Kaviriri *et al*., 2020; Khanova *et al*., 2020; Tuomainen *et al*., 2022). In previous studies on *P. sylvestris* and *P. koraiensis*, clonal seed orchards expected to express homogeneity in phenotypic traits, showed variations in cone and seed characters (Weng *et al.,* 2020). These characters were as follows: cone length, cone width, cone weight, apophysis (outer part of cone scale) length, apophysis width, number of filled seeds, total number of seeds, seed length, seed width, seed weights, opened carpel and total number of carpels (Bilir *et al*., 2008; Sevik & Topacoglu, 2015). Many factors have been attributed to poor cone opening such as: early harvesting, fungal and insect damage as well as case hardening during storage (Ayari & Khouja, 2014; Ayari *et al*., 2012, 2011; Bramlett, 1977).

Earlier studies stated that trees with best phenotypic traits are not necessarily the best seed producers (FAO, 1985). However, Bramlett (1977), also documented that the analysis of cone and seeds provides information that could enable breeders to evaluate seed production from seed orchards. Seed weight is an important indicator of seed quality since it positively correlates with seed germination rate and seedling size in conifers (Guemri *et al*., 2019; Maia & Corticeiro, 2022; Shen & Cho, 2021). Seed quality has largely been determined by seed physical (seed size and weight) and physiological traits (germination rate). Seed quantity is suggested to be enhanced by improving cone traits such as cone abundance per tree, number of seeds per cone and seed weight in an orchard (Grayson *et al*., 2002; Weng *et al*., 2020). The availability data on cone physical characteristics in relation to seed yield for *P. patula*, cone sorting guidelines and protocols could enhance germplasm production for this commercial plantation species.

2.7 Influence of pine seed extraction exposure periods on seed release

Seed extraction, storage, germination, and seedling growing protocols have been successfully developed and implemented at an operational level for producing thousands of seedlings for planting. Dry fruits such as pine cones require appropriate treatment for seed extraction (Belcher, 1977). The conventional way which is cheap and simple is by spreading the cones under the sun in drying beds until they open up for seed release. In other cooler regions of the world drying kilns and auxiliary mechanized sieving is used for cone drying and seed extraction. Certain attempts have been used to improve the pine seed extraction process as the natural sun drying procedure is very slow and depending on the weather it takes weeks to release the seeds. Most studies included trials of seed extraction in ovens at various temperatures. The duration of drying the cones reduced to about 4 days at specific temperatures normally higher, it could be reduced to 2.5 hours. Depending on the pine varieties, some seeds under these extraction temperatures did not show any significant deterioration as the germination percent remained highest. The seeds also exhibited lower moisture content and thus they could be stored for longer periods than the normal seeds.

A few pines have research work 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 open the scales of serotinous cones, and involve either heating cones in ovens or by immersion in water (Perry & Lotan, 1977; Reyes & Casal, 2001; Tapias *et al*., 2001). Previous studies have reported that pine cones open to release seed through a combination of temperature and humidity conditions which varies widely in the pine growing regions of the world (Aniszewska *et al*., 2020; Essl *et al*., 2011; Wyse *et al*., 2019). Another factor affecting pine cone opening and to some extent seed availability is the presence of resin with serotinous cones occurring in many species (Rhoades *et al*., 2022; Wyse *et al*., 2019). In these species, cone scales remain closed due to sealing with resin which require high temperatures for resins to melt and cones to open. The presence of resin is also considered as an adaptive defence mechanism against insect infestation to support growth and reproduction of the pine species (Farinha *et al*., 2018; Horstmann *et al*., 2022; Redmond *et al*., 2019).

Cone desiccation, without heating, has also been examined for *P. halepensis* (Wyse & Dickie, 2017) where it was confirmed that moisture levels had a crucial impact on cone opening for this pine species. In another study on cones from Aleppo pines clones, it was observed that cone moisture content did not vary significantly among the cones and that moisture content was predominantly under environmental control (Matziris, 1998). Seeds extraction from cones of many species requires drying either naturally or from artificial heat sources and a number of methods have been employed for seed extraction from such cones (Angaine *et al.*, 2021; Aniszewska, 2012; Onyango *et al.*, 2020).

Artificial drying of cones in heated kilns has been recommended for cool moist climate species where the climate in not suited for air drying (Ghildiyal *et al.*, 2008; Singh *et al.*, 2017). The major challenge associated with natural sun drying of cones is the possibility of losing viability due to moisture loss and temperature variation (Aniszewska & Zychowicz, 2020). Investigations to understand effects of temperature and moisture variations for seed release in *P. radiata* found no difference in seed release even when the cone moisture content was varied (Wyse *et al.*, 2019). In other studies, the use of microwave electromagnetic radiation in the first stage of hulling *P. sylvestris* cones reduced their initial moisture content, which resulted in quicker scale opening. The study established a relationship between loss of weight and the number of cones placed under a microwave generator. Thus, according to the study, the greater the number of cones, the lower the weight loss and the higher the quality of the extracted seeds. However due to the unevenness of heat in the microwave chamber, for industrial application, further investigation was recommended to ascertain the relationship between seed quality and microwave power and between the weight of the material by determining radiation flux (W/g) and unit irradiation (J/g) (Aniszewska *et al.*, 2019). Norway spruce seeds also demonstrated less resilience to microwave radiation as compared to European larch whose seed quality was retained on exposure to higher radiation power (Aniszewska *et al.*, 2024). Thermomechanical methods have been used in cone sorting and seed extraction in silvicultural extraction facilities within cooler countries (Novikov *et al.*, 2020). These methods have demonstrated greater yields in number of seeds suitable for long-term storage (Tulska *et al.*, 2022). The control of energy used in these processes optimised seed production and promoted sustainable use of natural resources (Tulska & Aniszewska, 2022).

Research findings have demonstrated that increasing summer temperatures lead to a greater incidence of *P. contorta* cone serotiny being broken by warm weather conditions in Southern Idaho (Knapp & Anderson, 1980). High temperatures cumulatively favour seed released over time. When considering efficiency in mechanized seed extraction, it is important to establish the extraction exposure duration at which seed yield is at its peak so as to focus the seed extraction efforts to the higher release periods. This is an area where the study sought to provide more information.

2.8 Influence of cone characteristics, seed extraction exposure periods and temperature on germination

Germination behaviour is shaped by external factors (biotic and abiotic), genes, or conditions experienced during seed maturation in the field. Together, these can determine, for instance, germination timing or emergence rate, survival effects and post germination traits (Bravo-Navas & Sánchez-Romero, 2022; Rajjou & Debeaujon, 2008). Seed germination is a sensitive but key process in the life cycle of pines and has an important impact on population expansion, seedling growth, establishment, individual survival and competition. 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 (Correia *et al*., 2014; Tonguç *et al*., 2022).

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 (Bramlett, 1977; Grayson *et al*., 2002). A number of pine species especially in fire prone areas have previously been investigated for the insulation capacity of cones for safe seed release (Calvo & Nunez, 2000; Habrouk *et al*., 1999; Koba & Zhigalova, 2019; Moya *et al*., 2007). It was observed that under different treatments, their respective cone responses to the temperatures and subsequent seed germination varied across the species tested (Bhat *et al*., 2017; Sivacioğlu & Ayan, 2008; Wyse *et al*., 2019). Gradual cone opening ranged from two to three hours after exposures to induced heat treatment for two of the pines *P. radiata* and *P. pinaster* (Bhat *et al*., 2017; Reyes & Casal, 2001; Sivacioğlu & Ayan, 2008; Wyse *et al*., 2019).

The capacity of cones to withstand high temperatures during cone opening to safely release under different temperature treatments was studied by Grayson *et al*. (2002) and Bramlett (1977) to understand the varied responses in three pines namely; *P. halepensis*, *P. sylvestris* and *P. nigra.* Tests on heat tolerance and regenerative capacity after fires of these species showed germination potential of *P. pinaster* and *P. nigra* after exposure to high temperature of 70-120°C and the variance between production in the scales. Observed regeneration patterns of P. nigra, P. sylvestris and P. halepensis after fire in large burned areas have been related to the interactions between their seeding phenology, cone resistance to opening, and the effects of fire severity on seed viability. *P. nigra* and *P. sylvestris* cones and seeds have exhibit a very low tolerance to fire effects, and thus regeneration have depended on the arrival of seeds from outside the boundaries of the burned area.

The high resistance displayed by *P. halepensis* cones to heat, as well as the resistance of their seeds to fire severity, allowed for its regeneration in burned areas. Thus spatial and temporal effects on the germination and regenerative capacity of these pine species played a role after forest fires (Habrouk *et al*., 1999). In addition, post fire resilience of *P. pallasiana* (pallasian pine) seeds and reproductive structures has been tested to understand the effects of seed weight and cones size on surviving the severity of fire in natural stands. Fire effect on the reproductive structures established that the flame damaging action does not cause total mortalities in seeds. In scorched cones it was discovered that vital functions of the seeds were partly preserved. The study also showed that, the chances of the seeds' survival following the heat shock strengthened with the cones' length thus seeds from bigger cones have maximum likelihood to sustain viability after the heat shock. This study recommended the selection of seed stock based on potential resilience to fire based on physical characteristics (Koba & Zhigalova, 2019).

Generally, serotinous cones and fruits are morphologically dehiscent but their dehiscence requires an exceptionally high temperature for resin to melt so that the scales open (Samano & Tomback, 2003; Schmidt, 2000) without losing the viability of seeds. Desiccation studies on two pine species (*Pinus resinosa* and *Pinus elliottii)* showed good germination performance in longer storage of both pine species. Researchers reported 86% germination in *P. resinosa* samples stored for 42 years (Bonner, 1990). Germination of *P. elliottii* was found to be 66% after 50 years of storage at 4°C, but there was evidence of loss of vigour. However, there exists opportunities for improvement where germination can be optimized under good environmental controls (Kanzler *et al*., 2014; Nel, 2002). 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 (Çetin, 2023).

Seed germinability and germination responses is neither temporally nor spatially the same for all the species (Bussmann & Lange, 2000). 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 (Reyes & Casal, 2001). Studies by Ghildiyal *et al*. (2008), 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 duration of exposure to thermal treatments (Lovreglio *et al*., 2007).

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 (Singh *et al*., 2017). Lodgepole pine seeds heated to temperatures over 80°C 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 (Knapp & Anderson, 1980).

Seed moisture content has been shown to fundamentally influence physiological reactions in seeds (Bautista *et al*., 2022; Bonner, 1990; Ghildiyal *et al*., 2008), and it follows that the tolerance of seeds to fire may differ during periods of higher or lower soil/seed moisture. Seeds that have been fully hydrated have demonstrated increased sensitivity to high temperatures such that seeds with high moisture content are at a higher risk of seed mortality when exposed to high temperature (Ruprecht *et al*., 2016). Thus, thermal tolerance of seeds greatly varies with seed moisture content (Tangney *et al*., 2019). For *P. patula*, 65°C has been recommended for rapid seed extraction as cones are set to open from 4 hours up to 24 hours (Onyango *et al*., 2020). However, seed germinability was not tested, thus, the safe extraction exposure period is yet to be established.

2.9 Contributing factors in pine cone and seed losses

a) *Pine cone damage and seed dormancy*

Cone and seed production, for a given season, also rely on the tree vigour, health size, stand condition, and the loss of seed through pests (*Leptoglossus occidentalis, Dioryctria mendacella*), diseases (*Fusarium circinatum*) or predation (squirrels and crossbill) (Barlow *et al*., 2007; Calama & Montero, 2007; Farinha *et al*., 2018; Mezquida & Benkman, 2021; Samano & Tomback, 2003; Tíscar, 2019). It has been established that the seed health in relation to seed production of lodgepole pines declines as the trees development process matures (de la Bastide *et al*., 2019). Fungal infections have been identified the major contributing factor to seed loses during germination (Bautista *et al*., 2022; Darge, 2017; Vivian *et al*., 2020).

Among factors affecting germination, the state of the seeds themselves is the most important. Some seeds might be dormant while others are not. Seed dormancy in pines could be physical, physiological or both. Different factors, alone or in combination, have been attributed to the dormancy in the different Pinus species (El-Kassaby *et al*., 2008; Fernando, 2014). Seedcoat inhibition has been suggested to play an important role in seed dormancy, and germination with removal of which showing improved germination for some species. Other causes of dormancy, in *Pinus lambertiana* (sugar pine), were related to under developed embryos; thus, special treatment such as cold stratification, chemical stratification, GA3 treatment, and artificial openings have been tried to break dormancy and improve germination (Shen & Cho, 2021).

An earlier study on P. clausa showed that older seeds extracted from older cones exhibited some dormancy as compared to seeds from younger cones. Viability was maintained when seeds were dried to 10% moisture, for 2 years storages at 25°F (-3.8°C) (Barnett & McLemore, 1966). In an experiment aimed at improving in *P. patula* germination, it was demonstrated that either soaking the seed in a 200 g/l polyethylene glycol 6000 solution for 15 days or soaking for 24 hours in a sea-weed concentrate followed by 20 day stratification at 2 to 3°C could safely reduce the dormancy percent of the seed (Donald, 1981).

b) *Influence of germination media and presowing environments on physiological process of pines*

Use of growth stimulants has been effective in reducing Siberian pines (*Pinus sibirica*) *Pinus sibirica* seed losses resulting from failure to develop full embryos. Plant growth media has been used as technology for improving seed propagation in terms of soil germination and seedling growth indicators of *P. sibirica* from different provenances. Stagnated embryo development in pines is mostly associated with inbreeding depression. These losses were quantified as the number of full seeds yielded per cone whereby inbreeding had affected the proportion of seeds with fully developed embryos but did not affect the numbers of extractable seeds. This technique shortened the technology for growing Siberian pines from seeds, as it did not require long-term stratification of seeds before sowing and also , excluded seed losses attributed to eating by rodents and birds (Karamysheva *et al*., 2019).

Scots pine seed germination and early growth in controlled conditions and in natural field conditions has been assessed to optimize overall germination performance (Sirgėdienė, 2019). Germination under field conditions was shown to be influenced by soil temperature, moisture, light intensity, seed colour, mass, seed source. However, in this study, site differences did not significantly affect seeds germination energy as it appeared to be quite similar indicating that even under harsh conditions seeds had equal possibilities to germinate. On the other hand, potting material and germination media was shown to influence early growth and germination of this species as increased levels of contamination in the planting media decreased the germination capacity and seedling growth (Makhniova *et al*., 2019).

Initial state of seeds and seed storage conditions have qualified longevity of seeds (Baah-Acheamfour & Sobze, 2022). Some studies in China have established that for long-term storage of *Pinus dabeshanensis* (white pine) seeds, 4℃ was effective in seed preservation, while if storage period is less than a year, room temperature storage was recommended emphasised for higher germination. Scarification before sowing for hard seed coated pines has also been encouraged in optimising germination because of the rapid water uptake (Maid *et al.*, 2019; Xiang *et al.*, 2019). *Pinus nigra* Arn. (Anatolian black pine) seeds have demonstrated significant and negative reactions to increased exposure time to UV-B radiation as the stress affected both germination and seedlings development (Ozel *et al.*, 2021).

2.10 Research gaps

Research gaps of the current study are shown in Table 1.

Table 1: Research gaps based on extent of previous work

Author and year of publication	Research topic	Research Gaps
Onyango *et al*. (2020)	Patula pine (*Pinus patula*) cones opening under different treatments for rapid seed extraction in Londiani, Kenya	The study focused on cone pretreatment and higher extraction temperatures for reduced seed extraction time. Seed germination was not part of the study.
Angaine *et al*. (2021)	Influence of *Cupressus lusitanica* Mill. Cones and Seed Characterization on Germination in Kenya	The study focused on improving seed sorting technique by cone size sorting, use of seed size and relative density to sort viable seeds. Fitness of seeds throughout the 24 hours extraction periods was not examined.
Fierro-Cabo and Plamann (2021)	Enhancing the seed germination process of Montezuma cypress (*Taxodium mucronatum* Ten.)	The study focused on improving germination by soaking and stratification of stored seed. Germination conditions was not part of the study.

2.10 Theoretical Framework

Forest fires and crown fires in pines are traditionally perceived as adverse phenomena capable of initiating a significant transformation on the forest community. The evolutions of pines have recognized the role of heat in the form of fire as influential in the succession and establishment of pines (Tapias *et al*., 2016). Pines have developed traits and diversified in extreme environments where other higher plants could not survive. These traits include the following: adaptation to drought, the capacity to close stomata on hot and dry days and at high water potentials, various ruderal strategies, vigorous regeneration, highly competitive abilities, and reduced growth and acclimatization during drought (Fulé *et al*., 2021; Lamont *et al*., 2020; Richardson *et al*., 1994). Traits such as enormous seed pressure, height, fast-growing, ectomycorrhiza, or thick bark contribute to the survival and dominance of pines. Strategic allocation of resources to plant reproduction or defence at the expense of other fitness traits has been a central component of plant life history theory of the pines. Adaptations to fire such as serotiny, self- pruning of dead branches, long needles, and the grass stage has also helped pine to survive and flourish in fire prone environments (Habrouk *et al*., 1999; Singh *et al*., 2018).

Diversification of pines have previously fallen into either fire-adapted and fire-avoider lineages. In mixed forests, fire tolerant pines such as, *Pinus attenuata*, *P. halepensis*, *P. brutia*, just to mention a few, outcompete oaks due to their vigorous postfire recruitment and eventually attain greater heights and thus overshadow the shorter statured oak. Rather than merely tolerating fire, these pines embrace fire by both enhancing the capacity for fires to consume tree canopies as well as delaying reproduction to a single postfire pulse of recruitment from serotinous cones (Keeley, 2012). The ecology of pines therefore has demonstrated a combination of two principle strategies, anchored on Charles Darwin's theory of survival for the fittest, to enhance survival in the natural environment: adaptations to ecological disturbance brought about by various fire regimes; and adaptations to extreme conditions (He *et al*., 2012).

In these theories, fire disturbance has favoured pine expansion by facilitating cone bursting and stimulating germination especially for regenerations (Ganatsas *et al*., 2008; Tapias *et al*., 2001, 2016). Fire intensity and frequency has acted as a factor of natural selection in pine species populations' regeneration and succession (Koba & Zhigalova, 2019). Morphological and physiological adaptations of pines to fire frequency and intensity vary across the species and regions where they are found (Essl *et al*., 2011; Ghildiyal *et al*., 2008). These ecological and life history characteristics ensure the retention of diversity through long life spans, large individual size, predominant outcrossing mating systems, large and relatively regular pollen and seed crops routinely distributed over great distances (primarily wind dispersed), and large, continuous populations. Expansion and invasion of pines is often limited until the onset of favourable conditions. Colonization and succession and invasion of *Pinus contorta* and *Pinus strobus* in Chile attribute their success to conservation of reproductive characteristics such as abundant seed rain in open favourable environments, production of heavier seeds, spiky seed dispersal, higher plant height, and variation in fruit types for these shade intolerant varieties (Singh *et al.*, 2018).

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 (Aniszewska *et al.*, 2019, 2020; Calvo & Nunez, 2000; Lovreglio *et al.*, 2007). Heat tolerance and thermal shock resistance as indicated by germination energy is shown to be dependent on species, cone and seed moisture content (Ghildiyal *et al.*, 2008; Habrouk *et al.*, 1999; Tangney *et al.*, 2019). 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.

2.11 Conceptual Framework

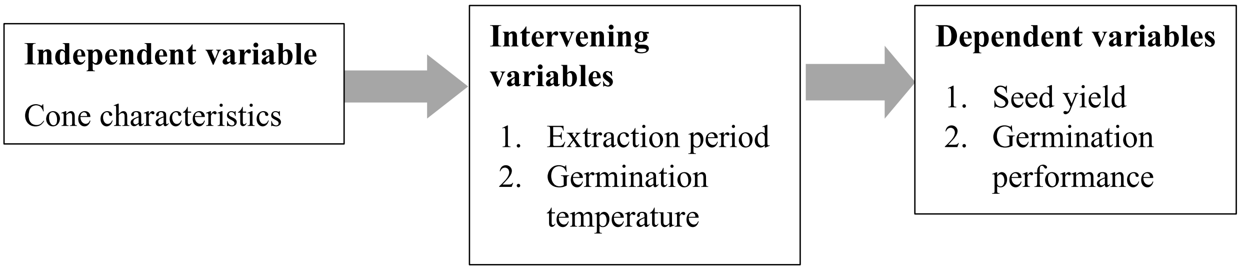
It has been argued that the differences in cone maturity, size, shape and weight influences the seed number per cone, seed potential, seed efficiency, seed morphology and seed germination behaviour of some pines (Angaine *et al.*, 2020; Aniszewska, 2010; Ganatsas *et al.*, 2008). Deflection of scales during cone opening for seed release is diverse depending on the cone shape (tapered or cylindrical), resin thickness, and moisture content (Aniszewska, 2010; Aniszewska & Bereza, 2014; Singh *et al.*, 2017). A summary of experimental variables captured in this study are shown in Table 2.

Table 2: Summary of experimental variables in the study

Independent variables	**Intervening variables**	**Dependent variables**
1.Cone size (width)	1.Extraction exposure periods	1. Quantity of seed released
2.Cone weight	2.Germination temperature conditions	2. Quantity of seed germinated

Figure 1 illustrates the scope of the study and possible determinants of seed germination performance based on the cone characteristics (narrow, wide, heavy and light), temperature exposure periods during extraction (6 hours, 12 hours and 24 hours), and germination temperature (22°C, 27°C and 32°C).

Figure 1: Conceptual framework



CHAPTER THREE
MATERIALS AND METHODS

3.1. Study site description

Londiani area is in the Rift Valley highlands of Kenya positioned between 35.607270°E longitude and 0.155520°S latitude (Figure 2).

Climate: Londiani area is cool and moist almost throughout the year with a minimum temperature of 8.6°C and a maximum of 23.31°C and an average temperature of 15.7°C. Londiani forest ecosystem landscape ranges from hilly to moderate terrain with altitude ranging from 1970 to 2900 m.a.s.l. The area has two rainy seasons, with the long rains occurring in the months of March to May with an average rainfall of 750 mm for the three months, and the 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 (KFS, 2018).

Geology and soils: The geology and soil types are influenced by ancient volcanic activities. The underlying rock is volcanic but varies according to its age. In general, the area is dominated by soils that have been developed from ashes and other pyroclastic rocks of recent volcanoes. The soils are predominantly dark reddish brown to dark red clays, classified as humic nitisols and humic cambisols (KARI, 1996).

Hydrology: Londiani Forest is part of the larger Mau Forest Complex ecosystem, which is of major economic and ecological importance in Kenya due to its value as a watershed and catchment area.

Vegetation: There is variation in vegetation cover and composition in the indigenous forest. In plantations forests, planting of monoculture plants is being practiced for production of wood suitable for processing by wood-based industries. The plantation species include; pines, cypress and eucalypts. Vegetation zones and species distribution depend on density variations of the particular tree species, topography, soil depth and human interference. Domination of some species in different parts of the forest has resulted in the development of different vegetation zones within the forest. Some of the important indigenous species found in the forest include; *Prunus africana, Podocarpus falcatus, Osyris lanceolata, Olea hochstetteri*, *Juniperus procera, , Olea africana, , Rhus natalensis, Warburgia ugandensis,* (KFS, 2018).

The factors identified to have contributed to the changes in forest cover and land use in Londiani forest include agriculture, human settlements, timber harvesting, extraction of building materials by the locals, grazing, fuel wood cutting and charcoal burning (Omondi & Musula, 2011; Ongong'a, 2012).

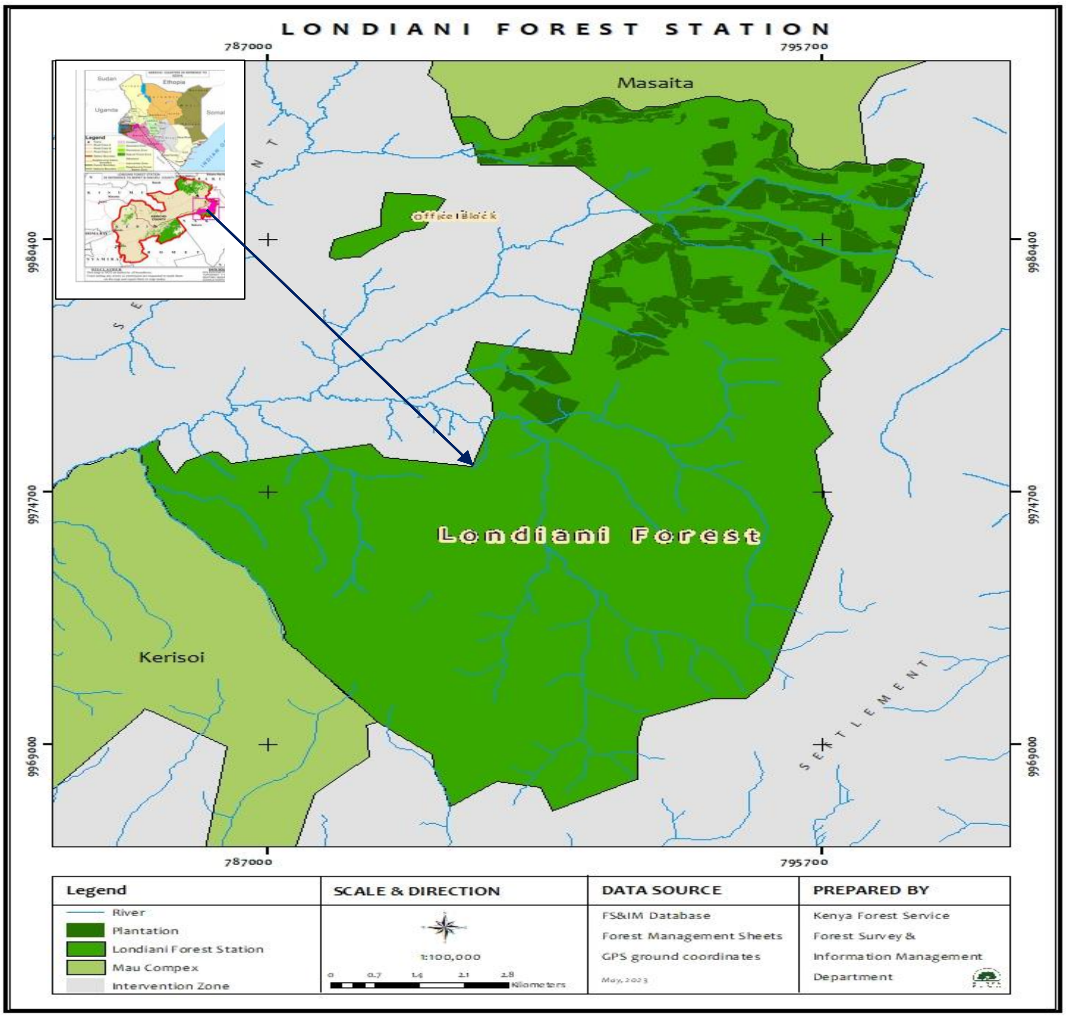


Figure 2 : Map of Londiani Forest, Source: KFS, (2023)

3.2. Sampling frame

3.2.1 Identification of sample trees

Sample trees for cone collection were selected from a 2006, *P. patula* clonal seed orchard. The seed source was situated in the Kamara block of Londiani forest. 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 after the first tree was randomly picked. The trees were marked and using a diameter tape, the diameter at 1.3 meters above ground, referred to as diameter at breast height (dbh) of each sampled tree was measured.

3.2.2. 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. Given that the maturity age of cones has been found to have a bearing on the opening temperature in some species, such as *P. halepensis* (Tapias *et al*., 2001), observation of cone colour (shiny brown to silver grey) was the criteria for determining mature cones of the current season. The cones were packed in gunny bags for delivery to KEFRI Londiani laboratory.

3.2.3. Data collection on cone characteristics and seed extraction

Once in the laboratory, 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 (Angaine *et al*., 2020). These cones were then exposed to artificial extraction conditions in ovens set at 65°C (Onyango *et al.,* 2020).

Observations of the seed released were done after 6, 12, and 24 hours. The categories under investigation were; Light, Heavy, Narrow, and Wide cones denoted as L, H, N, and W respectively with alphanumeric 1,2,3 representing extraction exposure periods; 6, 12, and 24 hours. Whereas exposure periods and cone characteristics were treatments, the variable was the seed released within the periods.

3.3 Experimental design

The experimental design used was completely randomised factorial 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 is illustrated in Figure 3 as follows:

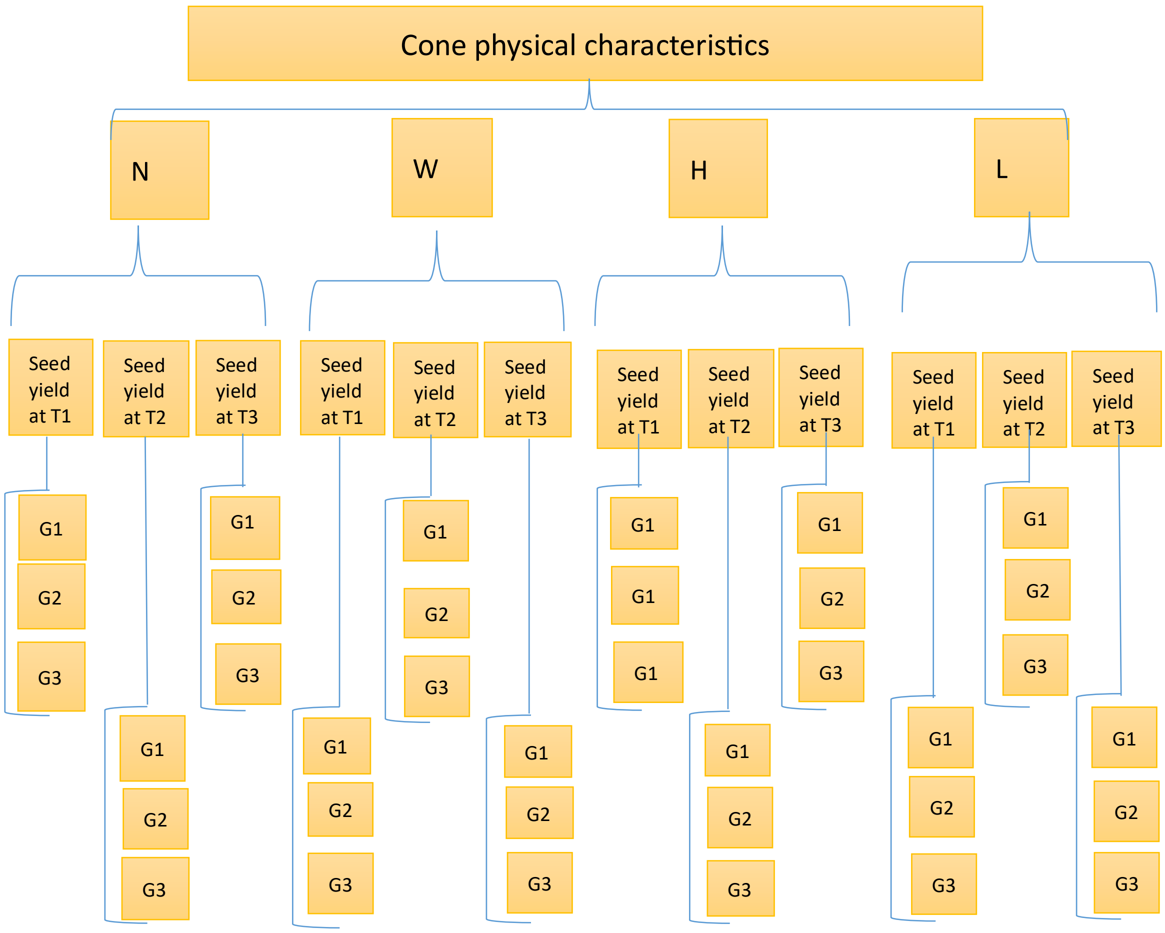


Figure 3: Sketch of study design. Source: Onyango A.A

Extracted seed was cleaned by removing the empty wings and dewinging the full seed by placing the seeds between the palms, rubbing against each other and gently blowing. Two representative samples of 10 seeds per group were subjected to moisture content tests. The constant temperature oven drying method (ISTA) was used by following the steps outlined: i) the glass petri dishes were sterilised at 130ºC (Memmert oven D2800) for one hour and allowed to cool in the desiccator for one hour ii) each petri dish was labelled and weighed, including the lid, and weights were recorded on the data sheet iii) two sub-samples of 10 randomly selected seeds from each group were placed into two separate petri dishes, which served as two replicates iv) the Petri dishes were placed with the lids removed in an oven maintained at 105°C (Memmert oven D2800) v) the seeds were dried for 17±1 hours vi) at the end of the drying period the lid on each petri dish was replaced vii) the petri dishes were moved to a desiccator and allowed to cool for 45 minutes viii) the weight of the petri dishes containing the samples were recorded.

The moisture content was calculated on a wet-weight basis and expressed as a percentage using the following formula:

$$Moisture\ content\ (\%) = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where;

W_1 = weight of petri dish with lid;

W_2 = weight of petri dish with lid and sample before drying;

W_3 = weight of petri dish with lid and sample after drying.

Another sample of 10 seeds per group were subjected to the cutting test. This was done by nipping the seed longitudinally using a nail clipper for visual inspection of the embryo. Germination for seeds released from each group was carried out in germination chambers at fixed temperatures; 22°C (Sanyo growth cabinet MLR 350), 27°C (Panasonic MLR-352H-PE), and 32°C (LMS Cooled incubator model 600) for four weeks (Aniszewska & Bereza, 2014).

In preparation for seed sowing, petri dishes were sterilized and a thin layer of moist cotton wool was used as the germination media. Each petri dish had 100 seeds replicated three times from each of the extraction treatments (ISTA, 2023). Radicle emergence was used as the criterion for germinability (Ghildiyal *et al.*, 2008; ISTA, 2011). The number of germinated seeds were recorded daily for up to day 28 with those counted being discarded (counting without replacement). Observations of germination by radicle emergence was indicative of seed vigour for seeds exposed to the different controlled conditions.

Treatment combinations (36) and replicates (3) are shown in Table 3

Table 3: Treatments and replicates

Treatment number	Treatments	Replicates		
		REP 1	**REP 2**	**REP 3**
1.	HT_1G_1	HT_1G_{11}	HT_1G_{12}	HT_1G_{13}
2.	LT_1G_1	LT_1G_{11}	LT_1G_{12}	LT_1G_{13}
3.	HT_1G_2	HT_1G_{21}	HT_1G_{22}	HT_1G_{23}
4.	LT_1G_2	LT_1G_{21}	LT_1G_{22}	LT_1G_{23}
5.	HT_1G_3	HT_1G_{31}	HT_1G_{32}	HT_1G_{33}
6.	LT_1G_3	LT_1G_{31}	LT_1G_{32}	LT_1G_{33}
7.	HT_2G_1	HT_2G_{11}	HT_2G_{12}	HT_2G_{13}
8.	LT_2G_1	LT_2G_{11}	LT_2G_{12}	LT_2G_{13}
9.	HT_2G_2	HT_2G_{21}	HT_2G_{22}	HT_2G_{23}
10.	LT_2G_2	LT_2G_{21}	LT_2G_{22}	LT_2G_{23}
11.	HT_2G_3	HT_2G_{31}	HT_2G_{32}	HT_2G_{33}
12.	LT_2G_3	LT_2G_{31}	LT_2G_{32}	LT_2G_{33}
13.	HT_3G_1	HT_3G_{11}	HT_3G_{12}	HT_3G_{13}
14.	LT_3G_1	LT_3G_{11}	LT_3G_{12}	LT_3G_{13}
15.	HT_3G_2	HT_3G_{21}	HT_3G_{22}	HT_3G_{23}
16.	LT_3G_2	LT_3G_{21}	LT_3G_{22}	LT_3G_{23}
17.	HT_3G_3	HT_3G_{31}	HT_3G_{32}	HT_3G_{33}
18.	LT_3G_3	LT_3G_{31}	LT_3G_{32}	LT_3G_{33}
19.	WT_1G_1	WT_1G_{11}	WT_1G_{12}	WT_1G_{13}
20.	NT_1G_1	NT_1G_{11}	NT_1G_{12}	NT_1G_{13}
21.	WT_1G_2	WT_1G_{21}	WT_1G_{22}	WT_1G_{23}
22.	NT_1G_2	NT_1G_{21}	NT_1G_{22}	NT_1G_{23}
23.	WT_1G_3	WT_1G_{31}	WT_1G_{32}	WT_1G_{33}
24.	NT_1G_3	NT_1G_{31}	NT_1G_{32}	NT_1G_{33}
25.	WT_2G_1	WT_2G_{11}	WT_2G_{12}	WT_2G_{13}
26.	NT_2G_1	NT_2G_{11}	NT_2G_{12}	NT_2G_{13}
27.	WT_2G_2	WT_2G_{21}	WT_2G_{22}	WT_2G_{23}

Treatment number	Treatments	Replicates		
		REP 1	REP 2	REP 3
28.	NT_2G_2	NT_2G_{21}	NT_2G_{22}	NT_2G_{23}
29.	WT_2G_3	WT_2G_{31}	WT_2G_{32}	WT_2G_{33}
30.	NT_2G_3	NT_2G_{31}	NT_2G_{32}	NT_2G_{33}
31.	WT_3G_1	WT_3G_{11}	WT_3G_{12}	WT_3G_{13}
32.	NT_3G_1	NT_3G_{11}	NT_3G_{12}	NT_3G_{13}
33.	WT_3G_2	WT_3G_{21}	WT_3G_{22}	WT_3G_{23}
34.	NT_3G_2	NT_3G_{21}	NT_3G_{22}	NT_3G_{23}
35.	WT_3G_3	WT_3G_{31}	WT_3G_{32}	WT_3G_{33}
36.	NT_3G_3	NT_3G_{31}	NT_3G_{32}	NT_3G_{33}

Combinations for one replicate are 36. So 3 replicates gave 36×3=108 experimental units or plots

KEY:

H= Heavy cone

L= Light cone

N= Narrow cone

W= Wide cone

T_1= oven exposure period 6 hours

T_2= oven exposure period 12 hours

T_3= oven exposure period 24 hours

G_1= Germination chamber at 22°C

G_2= Germination chamber at 27°C

G_3=Germination chamber at 32°C

Germination was considered as percentage of the seed yield resulting from treatments, H, L, W, N and T. Thus, germination performance was the resultant effect of the interaction effect of cone characteristics and extraction exposure period and the germination chamber conditions. These were analysed according to ISTA (2023) procedures,

Where;

$$y = (no\ of\ geminated\ seeds\ \div no\ of\ seeds\ sown) \times 100\%$$

Seed samples from each group were sown in the nursery to simulate nursery practice and observe early growth behaviour. Sand in the seed sowing bed was sterilized by use of sodium hypochlorite solution (JIK 3.5%) mixed with water in the ratio of 1:100. Tags were placed in the uniformly partitioned seed bed and labelled with the different seed lot treatments. Seed samples were sprinkled with a spatula, covered with little sand and the bed was kept moist.

3.4 Statistical analysis

Data on initial cone width, initial cone weight, cone opening period, cone seed count and seed germination was collected and tabulated in a data sheet using MS excel. Descriptive statistics was applied to analyze the cone composition and germination trends 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 determine significant differences in means between groups at p=0.05. Tukey HSD was the post-hoc test of choice to separate the overlaps in means between the treatments under study.

Correlation coefficients were categorized as weak (0.0–0.4), moderate (0.4–0.6) and strong (0.6–1.0) (Mubai *et al*., 2020). Statistical data analysis for each specific objective are shown in Table 4.

Table 4: Statistical data analysis for each objective

Research Objectives	Variables	Statistical data analysis method
1. To determine the effect of cone width and exposure period on seed release.	**Independent variables** Cone width Wide cones Narrow cones Extraction periods 6 hours 12 hours 24 hours **Dependent variables** 1. Number of seeds released	-Descriptive statistics tables, graphs -Pairwise Comparisons F test
2. To determine the effect of cone weight and exposure period on seed release.	**Independent variables** Cone width Heavy cones Light cones Extraction periods	-Descriptive statistics tables, graphs -Pairwise Comparisons F-test

Research Objectives	Variables	Statistical data analysis method
	6 hours 12 hours 24 hours **Dependent variables** - Number of seeds released	
3. To analyze the correlations between cone width, exposure period and germination temperature on seed germination.	**Independent variables** Cone width Wide cones Narrow cones Exposure periods 6 hours 12 hours 24 hours Germination temperature 22℃ 27℃ 32℃ **Dependent variables** -Number of seeds germinated	-Trends graphs -ANOVA, Post hoc test (Tukey HSD) -Pearsons's correlation analysis -Regression analysis
4. To analyze the correlations between cone weight, exposure periods and germination temperature on seed germination.	**Independent variables** Cone weight Wide cones Narrow cones Exposure periods 6 hours 12 hours 24 hours Germination temperature 22℃	-Trends graphs -ANOVA, Post hoc test (Tukey HSD) -Pearsons's correlation analysis -Regression analysis

Research Objectives	Variables	Statistical data analysis method
	27℃ 32℃ **<u>Dependent variables</u>** -Number of seeds germinated	

CHAPTER FOUR

RESULTS

4.1 Effect of cone width and exposure period on seed release.

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 of seed extraction (Figure 4). Overall, there were significant differences (p=0.001) within the groups based on extraction period and cone physical characteristics.

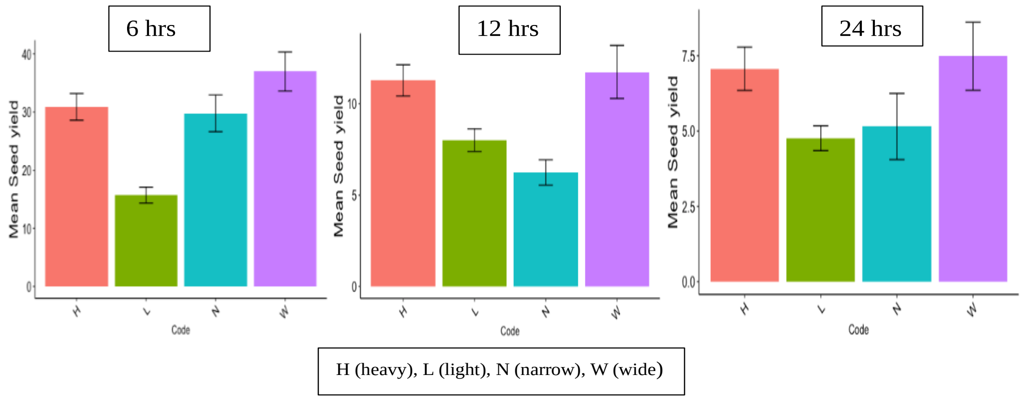


Figure 4:Mean seed yield at the end of extraction periods; 6, 12 and 24 hours, based on the cone characteristics

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 F=6.0118 (F_{crit}=1.5458), (Appendix 6) whereas heavy and wide cones showed no significant differences within this extraction period at p=0.24. F test between groups revealed a significant difference in seed yield as a result of cone width as compared to cone weight (p=0.001).

4.2 Effect of cone weight and exposure period on seed release

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 (Figure 5). 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.

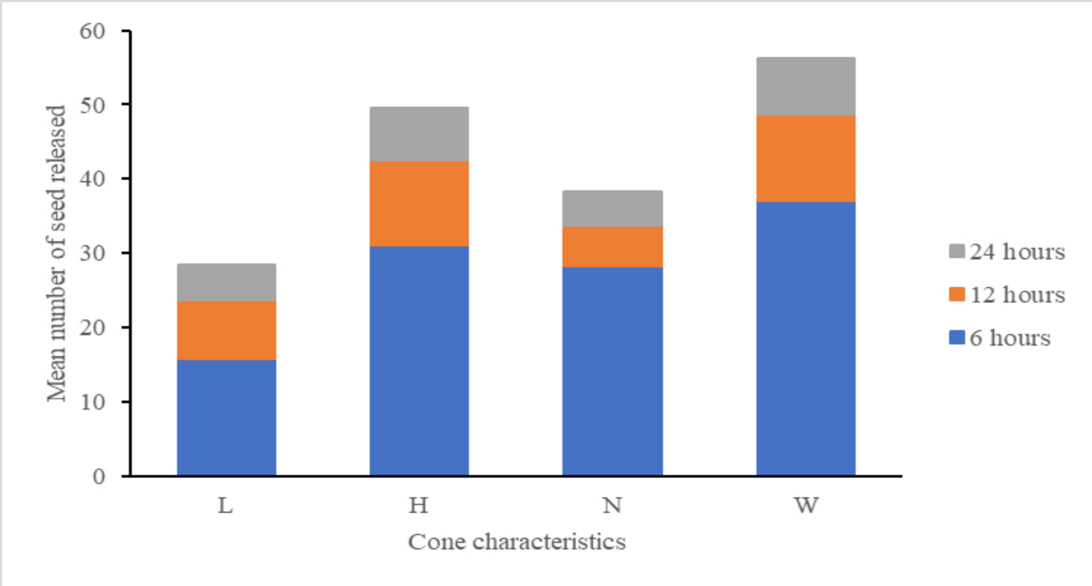


Figure 5: Seed release corresponding to extraction exposure periods and cone characteristics

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 (Figure 6). 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.

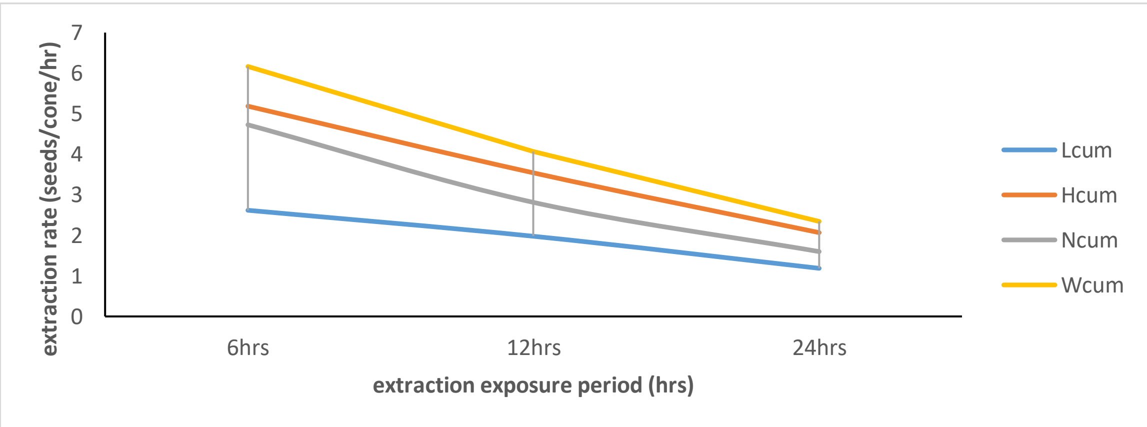


Figure 6: Extraction rates of seeds from Light (L), Heavy (H), Narrow (N) and Wide (W) cones

4.3 Correlations between cone width, exposure period and germination temperature on seed viability.

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. Similarly, germination temperature also showed weak negative but significant relationships with germination performance in this narrow cone category (Table 5).

Table 5: 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 p≤ 0.05 level; ns: p>0.05 correlation not significant

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℃ (Appendix 9).

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 6).

Table 6: 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

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 with F=16.415 (F_{crit}=4.757), p=0.0027 the seeds showing better germination performance at the three chamber temperatures in the wide cones category were from cones exposed to extraction for 24 hours without breaks.

4.4 Correlations between cone weight, exposure period and germination temperature on viability.

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. Germination performance and germination temperature correlated positively, weakly and insignificant (Table 7).

Table 7: 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 p≤ 0.05 level; ns: p>0.05 correlation not significant

Seeds exposed to the three extraction periods from heavy cones showed weak but positive interaction with germination performance. This weak positive correlation was not statistically significant (Table 8). For this group the germination performance negatively correlated with germination temperature conditions but it was also not significant.

Table 8: Pearson correlation coefficient for germination of seeds from heavy cones

Variables	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

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 (Appendix 9). 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 °C 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.

CHAPTER FIVE

DISCUSSION

5.1 Influence of cone width and exposure period on seed release.

There was difference in seed yield as a result of cone characteristics and extraction exposure duration. This concurs with previous case 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 (Ganatsas *et al*., 2008; Matziris, 1998; Weng *et al*., 2020). 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 (Sevik & Topacoglu, 2015; Bilir *et al*., 2008). Seed production from seed orchards has been suggested to be enhanced by improving cone traits such as cone abundance per tree, number of seeds per cone and seed weight (Fernando, 2014; Hauke-Kowalska *et al*., 2019).

Taking into consideration, cone width as a measure of cone size, the findings indicated that wide cones released more seed cumulatively and also demonstrated better initial seed release quantities. The current findings corroborate with previous work (Angaine *et al*., 2020; Dylewski *et al*., 2021; Nieto De Pascual-Pola *et al*., 2003) that, other factors notwithstanding, the size of cones is positively correlated with seed yield.

Pinus densiflora cones produced across different geographic and climatic zones in Japan showed variations in cone characteristics (cone and seed size, seed production and productivity per cone) (Iwaizumi *et al*., 2019). In these natural populations, cone size (length width and mass) was shown to influence seed productivity (number of filled seeds per cone, seed mass per cone). Across the sites, it was noticed that the larger the cone size, the higher the seed productivity per cone and thus the higher resource use efficiency. These findings are in agreement with the current study. Better productivity from bigger cones across the environments insinuated that the larger the cones, the higher the elasticity to environmental changes (Iwaizumi *et al*., 2019). Some authors for *Pinus thunbergii*, *P. roxburghii*, and *P. canariensis* have suggested that it is more adaptive and worth the investment to produce a limited number of larger cones and at specific crown positions for optimal growing rather than producing excess cones that contribute little to the reproductive success (Iwaizumi *et al*., 2021).

5.2 Influence of cone weight and exposure period on seed release.

The results from this study showed that there was a positive effect of exposure period on seed release cumulatively. An increase in extraction exposure period, regardless of the cone's physical characteristics, ultimately resulted to increased seed output. High temperatures cumulatively favour seeds released over time (Garcillán, 2010; Romero & Ganteaume, 2020; Wyse *et al*., 2019). Notably, the extraction rate was optimal within the first six hours as the individual cone seed output was observed to be highest within this extraction period. Low extraction rate (0.4 seeds per cone per hour) and cumulative low seed numbers were observed for narrow cones at the end of 24 hours under controlled conditions.

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 (Ayari *et al*., 2016; Ayari & Khouja, 2014; Horstmann *et al*., 2022; Matziris, 1998). There were instances of cone inverse opening with increase in exposure duration where the scales deflected downwards towards the scar. 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. Another possible explanation was the natural changes of temperature and humidity in the air that might have facilitated reclosing of cones as experienced in *Pinus halepensis* (Singh *et al*., 2017).

Delays in *P. patula* and *P. radiata* cones opening due to moisture levels had been shown to be less significant in previous studies, showed temperature had a significantly greater influence on cone opening than moisture content (Onyango *et al*., 2020; Wyse *et al*., 2019). In addition to cone dimensions, serotinous species have been shown to experience challenges in opening even in the presence of fire mostly attributed to genetic attributes referred to the resin bonding strength (Lamont *et al*., 2020). Thus, this study could not rule out the possibility of the delays experienced especially in narrow cones being linked to similarities in genes.

Results from this study identified seed number as dependent on cone characteristics, and their viability as an independent variable and unpredictable from the outward appearance of the cone. The highest seed yield did not necessarily mean the highest germination percent. However, based on cone characteristics such as width and weight, seed yield and germination could be inferred as wider cones yielded more seeds and better germination potential was from heavy cones. Cones of *P. sylvestris* have been characterized on the basis of differences in the ability to adapt to changes in environment and intensity of cone production.

According to the current study, the differences in the selected parameters contributed to the dissimilarities in reproductive processes, survivability, growth and development energy within the pine stand (Neyko *et al*., 2020). In other studies, investigating the effects of inbreeding depression on seed production in Scots pine, it was discovered that while the numbers of extractable seeds were unaffected by inbreeding, the proportion with fully developed embryos (full seeds) was strongly affected. This demonstrated that the levels of abortion and germination was affected by levels of self-fertilization; as the yield of full seeds per cone declined with increased self-fertilization (Mullin *et al*., 2019). Therefore, the lower yielding cones in this study, in terms of number of seeds per cone could have suffered some genetic depression.

5.3 Correlations between cone width, exposure period and germination temperature on seed viability

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) whereby as the extraction time increased the number of seeds germinated reduced at warmer chamber temperatures.

The trends in the narrow cones category showed 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 (Bae & Kim, 2020; Koba & Zhigalova, 2019).

The current research, observed differences in seed coat colour which ranged from light brown to dark brown seeds and the shades were mostly consistent with the cone colour, where light brown seeds were from lighter shade of brown cones. Selection of forest tree seeds for nursery use focused on obtaining seed material with the expected biological characteristics has been done using the correlation of seed germination capacity based on their physical properties such as seed coat colour, mass, density, size, shape, aerodynamic characteristics, coefficient of friction, surface textures (Batkhuu *et al*., 2020; Kaliniewicz *et al*., 2014; Udval & Batkhuu, 2013).

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 (Novikov *et al*., 2021). 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 seedlot for commercial use, but germination rate influences the uniformity of emergence in nurseries (El-Kassaby *et al*., 2008; Yousefpour *et al*., 2017).

Previous studies have shown that both environmental and genetic factors influences the potential of trees to produce fertile quality seeds whereby depending on the trees 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 where the seeds are matured important role in determining a forest tree's potential for seed quality and seed fertility (Batkhuu *et al*., 2020; Beckman *et al.*, 2020; Guemri *et al*., 2019; Zhang *et al*., 2020).

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 (Correia *et al*., 2014). 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 (Maia & Corticeiro, 2022).

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 show that germination rates decline with increase in exposure period within the three phases of extraction. Exposure to higher seed extraction temperatures have been shown to negatively influence germination of pine seeds (Aniszewska *et al*., 2019).

5.4 Correlation between cone weight, exposure period and germination temperature on seed viability

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 (Barbour, 2007).

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 are also different. Moisture content has been shown to affect seed deterioration rate and has impacted on storage longevity of seeds (Mutai, 2018; Tangney *et al*., 2019). 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 durations phases at the constant temperatures 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 (Habrouk *et al.*, 1999; Lamont *et al*., 2020; Lovreglio *et al*., 2007).

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 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 (Bravo-Navas & Sánchez-Romero, 2022).

Numerous approaches have been devised in attempts towards improving technologies for assessing cone properties and seed germination capacity. These include; use of uv radiation (Ozel *et al*., 2021), alteration of storage conditions (Makhniova *et al*., 2019; Xiang *et al*., 2019), mathematical modelling of germination parameters (El-Kassaby *et al*., 2008; Omondi *et al*., 2020), use of x-ray radiography to determine fullness of seeds (Karamysheva *et al*., 2020; Nieto De Pascual-Pola *et al*., 2003), macro or micronutrients (nutripriming) or plant-based natural extracts as hydration media (Farooq *et al*., 2019) just to mention a few.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

All the four hypotheses were rejected as the study established significant influences of cone physical characteristics, extraction exposure periods and germination temperature on seed yield and subsequent seed germination performance of *P. patula*.

P. patula cones have demonstrated variations in cone weight and cone width from a single even aged seed source. Cone width had a greater influence on the amount of seed release than cone weight because of the significant differences in seed output of individual cones within the cones in the wide category. Wider cones consistent showed better and positive responses to heat in relation to the quantity of seed released throughout the extraction exposure phases. This could be explained as the advantage wide cones have in the scales flexibility; greater cone scales surface area for heat absorption, and more room for scale deflection going by cone shape.

Longer exposure durations led to cumulative increase in *P. patula* seed yield but the first few hours yielded optimum number of seeds. The first few hours played both functions of cone drying, resin melting and cone scale opening. A combination of processes such as the reduced cone moisture contents, weakened bonding strength within the scales by removal of the resin, and low surrounding (oven) air pressure due to heating causes the cones to give in by bursting. This under-pressure release explains the high numbers of seed released within the first few hours of extraction from the four cone groups.

Germination of seeds from big size cones showed better correlations with extraction exposure periods and germination temperature. *P. patula* cone weight showed greater influence on germination than cone width. Seeds produced from heavy 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.

The findings from this study informs the germination behaviour of freshly extracted pine seed based on cone characteristics, extraction durations and germination temperature conditions. Also, it can be applicable in handling and conservation of pine seeds in the wake of changes in environmental factors such as temperatures and rainfall. In the event, pine seeding season's change as a result of aging stands or climate change, germplasm producers could use cone characteristics to optimize seed collection and conduct breeding for seed production based on mother trees crown and cone characteristics.

6.2 Recommendations

i. It is recommended that seed extraction could be focused within six hours for energy conservation and improved efficacy in seed extraction through artificial heating.

ii. Germination under controlled temperatures showed consistency for recommendation of warmer temperatures between of 27℃ to 32℃ for higher germination performance.

iii. To enhance the germination performance of germplasm resources, cone sorting of *Pinus patula* for wider and heavier cones is recommended from improved seed extraction and germination potential.

iv. Further studies should be carried out on *P. patula* seed storage behaviour in relation to germination potential.

REFERENCES

Adegbehin, J. O. (1982). Growth and yields of Pinus patula in some parts of Eastern Africa with particular reference to Sao-hill, Southern Tanzania. *The Commonwealth Forestry Review*, *61*(1), 27–32. http://www.jstor.org/stable/42606126

Agne, M. C., Fontaine, J. B., Enright, N. J., Bisbing, S. M., & Harvey, B. J. (2022). Demographic processes underpinning post-fire resilience in California closed-cone pine forests: the importance of fire interval, stand structure, and climate. *Plant Ecology*, *223*, 751–767. https://doi.org/10.1007/s11258-022-01228-7

Alejano, R., Domínguez-Delmás, M., García-González, I., Wazny, T., Vázquez-Piqué, J., & Fernández-Martínez, M. (2019). The age of black pine (Pinus nigra Arn. ssp. salzmannii (Dunal) Franco) mother trees has no effect on seed germination and on offspring seedling performance. *Annals of Forest Science*, *76*(15), 1–10. https://doi.org/10.1007/s13595-019-0801-7

Angaine, P. M., Onyango, A. A., Ndungú, S. M., Inoti, S. K., & Owino, J. O. (2021). Influence of Cupressus lusitanica Mill. cones and seed characterization on germination in Kenya. *Journal of Forests*, *8*(2), 123–130. https://doi.org/10.18488/journal.101.2021.82.123.130

Angaine, P. M., Onyango, A. A., & Owino, J. O. (2020). Morphometrics of Pinus patula crown and its effect on cone characteristics and seed yield in Kenya. *Journal of Horticulture and Forestry*, *12*(3), 94–100. https://doi.org/10.5897/JHF2020.0635

Aniszewska, M. (2010). Analysis of opening cones of selected coniferous trees. *Annals of Warsaw University of Life Sciences, Forestry and Wood Technology*, 71(55), 57–64. http://annals-wuls.sggw.pl/files/files/afe2010no55full.pdf#page=57

Aniszewska, M. (2012). A method for seed extraction process in the common pine (Pinus sylvestris L .) subjected to mechanical processing by removal of stem. *Annals of Warsaw University of Life Sciences, Agricultural and Forest Engineering*, *59*, 107–113.

Aniszewska, M. (2013). Changes in humidity and temperature inside the pine cones (Pinus sylvestris L .) in two stages seed extraction. *Leśne Prace Badawcze (Forest Research Papers)*, *74*(3), 205–214. https://doi.org/10.2478/frp-2013-0020

Aniszewska, M., & Bereza, B. (2014). Analysis of water absorption process in the cones of common pine (Pinus sylvestris L .). *Annals of Warsaw University of Life Sciences, Agricultural and Forest Engineering*, *63*, 105–112.

Aniszewska, M., Gendek, A., Tulska, E., Peska, P., & Moskalik, T. (2019). Influence of the duration of microwave irradiation of scots pine (pinus sylvestris L.) cones on the quality of harvested seeds. *Forests*, *10*(1108), 1–15. https://doi.org/10.3390/F10121108

Aniszewska, M., Gendek, A., Tulska, E., Zięba-Kawecka, A., Malaťák, J., Tamelová, B., Malaťáková, J., & Krilek, J. (2024). Effects of electromagnetic waves on the moisture content of cones and the quality of extracted seeds in the Norway spruce and European larch. *New Forests*, *55*, 305–322. https://doi.org/10.1007/s11056-023-09979-5

Aniszewska, M., Zychowicz, W., & Gendek, A. (2020). The effectiveness of short-term microwave irradiation on the process of seed extraction from Scots pine cones (Pinus sylvestris L.). *IForest - Biogeosciences and Forestry*, *13*, 73–79. https://doi.org/10.3832/ifor3089-012

Ayari, A., & Khouja, M. L. (2014). Ecophysiological variables influencing Aleppo pine seed and cone production : a review. *Tree Physiology*, *34*, 426–437. https://doi.org/10.1093/treephys/tpu022

Ayari, A., Meftahi, M., Zammeli, F., & Khouja, M. (2016). Seed Production Variability of Aleppo Pine (Pinus Halepensis Mill.) within Korbus Arboretum (North East of Tunisia). *Global Journal Of Botanical Science*, *4*(1), 20–23. https://doi.org/10.12974/2311-858x.2016.04.01.3

Ayari, A., Moya, D., Rejeb, M. N., Ben Mansoura, A., Albouchi, A., De Las Heras, J., Fezzani, T., & Henchi, B. (2011). Geographical variation on cone and seed production of natural Pinus halepensis Mill. forests in Tunisia. *Journal of Arid Environments*, *75*(5), 403–410. https://doi.org/10.1016/j.jaridenv.2011.01.001

Ayari, A., Moya, D., Rejeb, M. N., Garchi, S., & de las Heras, J. (2011). Fructification and species conservation on Pinus halepensis Mill forests in Tunisia: Managing structure using individual tree size. *Pine Forests: Types, Threats and Management*, *May 2015*, 61–80.

Ayari, A., Zubizarreta-Gerendiain, A., Tome, M., Tome, J., Garchi, S., & 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*(1), 128. https://doi.org/10.5424/fs/2112211-11463

Baah-Acheamfour, M., & Sobze, J.-M. (2022). Assessing post-harvest interim seed storage conditions: a case study of four boreal plant species. *Seed Science and Technology*, *50*(2), 183–194. https://doi.org/10.15258/sst.2022.50.2.02

Bacherikov, I. V., Raupova, D. E., Durova, A. S., Bragin, V. D., Petrishchev, E. P., Novikov, A. I., Danilov, D. A., & Zhigunov, A. V. (2022). Coat Colour Grading of the Scots Pine Seeds Collected from Faraway Provenances Reveals a Different Germination Effect.

Seeds, *1*(1), 49–73. https://doi.org/10.3390/seeds1010006

Bae, H., & Kim, J. (2020). Functional Principles of Morphological and Anatomical Structures in Pinecones. *Plants*, *9*(10), 1343. https://doi.org/10.3390/plants9101343

Barbour, M. G. (2007). Closed-cone Pine and Cypress forests. In M. G. Barbour, T. Keeler-Wolf, & A. A. Schoenherr (Eds.), *Terrestial vegetation of California* (3rd ed., pp. 296–312). University of California Press. https://www.degruyter.com/document/doi/10.1525/9780520933361-013/html

Barlow, J., Mestre, L. A. M., Gardner, T. A., & Peres, C. A. (2007). The value of primary, secondary and plantation forests for Amazonian birds. *Biological Conservation*, *136*(2), 212–231. https://doi.org/10.1016/j.biocon.2006.11.021

Barnett, J. P., & McLemore, B. F. (1966). Sand Pine cones and seed. In *Tree Planters' Notes, USDA* (Vol. 76). https://www.srs.fs.usda.gov/pubs/misc/ja_1966_barnett_002.pdf

Batkhuu, N.-O., Udval, B., Jigjid, B.-E., Jamyansuren, S., & Fischer, M. (2020). Seed and Cone Morphological Variation and Seed Germination Characteristics of Scots Pine Populations (Pinus sylvestris L.) in Mongolia. *Mongolian Journal of Biological Sciences*, *18*(2), 41–54. https://doi.org/10.22353/mjbs.2020.18.14

Bautista, A. A., Vázquez, J. L. A., Badillo, M. E. V., García, A. R., Uribe, L. A. A., Juárez, A. H., Ángel, E. C.-D., & Hernández, J. M. (2022). Correlation between Relative Humidity and Forest Seeds Moisture on the Incidence of Fungi. *International Journal of Forestry Research*, *2022*, 1–10. https://doi.org/10.1155/2022/7917704

Beckman, N. G., Aslan, C. E., Rogers, H. S., Kogan, O., Bronstein, J. L., Bullock, J. M., Hartig, F., HilleRisLambers, J., Zhou, Y., Zurell, D., Brodie, J. F., Bruna, E. M., Cantrell, R. S., Decker, R. R., Efiom, E., Fricke, E. C., Gurski, K., Hastings, A., Johnson, J. S., … Zambrano, J. (2020). Advancing an interdisciplinary framework to study seed dispersal ecology. *AoB PLANTS*, *12*(2), 1–18. https://doi.org/10.1093/aobpla/plz048

Belcher, E. . (Ed. . (1977). The processing of conifer seed. *Small Lot Forest Seed Processing Workshop: October 18-20*, 9–17. https://www.google.com/books?hl=en&lr=&id=yxIcoXeE8uUC&oi=fnd&pg=PA2&dq=The+processing+of+conifer+seed+Belcher&ots=s5gkfEs5pG&sig=EPUNtnVYpwOimLGYmlxsry1L-4c

Bhat, G. M., Mughal, A. H., Malik, A. R., & Islam, M. A. (2017). Developing a New Technique for Rapid Seed Extraction from the Cones of Himalayan Conifer (Pinus Wallichiana A.B. Jackson). *Chem Sci Rev Lett*, *6*(22), 746–751. https://chesci.com/wp-content/uploads/2017/01/V6i22_17_CS212048033_Mohammad_746-751.pdf

Bialobok, S., & Zelawski, W. (1976). *Outline of physiology of Scots pine.: Vol. TT 73-5408* (S. Białobok & W. Żelawski (eds.)).

Bilir, N., Prescher, F., Lindgren, D., & Kroon, J. (2008). Variation in cone and seed characters in clonal seed orchards of Pinus sylvestris. *New Forests*, *36*, 187–199. https://doi.org/10.1007/s11056-008-9092-9

Bonner, F. T. (1987). Cone storage and seed quality in Longleaf Pine. In *Research note - USDA* (pp. 1–4). https://doi.org/10.1177/016555158501100107

Bonner, F. T. (1990). Storage of seeds: Potential and limitations for germplasm conservation. *Forest Ecology and Management*, *35*(1–2), 35–43. https://doi.org/10.1016/0378-1127(90)90230-9

Bramlett, D. L. (1977). Efficiency of Seed Production in Southern. *Thirteenth Lake States Forest Tree Improvement Conference*. https://www.fs.usda.gov/treesearch/pubs/14783

Bravo-Navas, M. V., & Sánchez-Romero, C. (2022). Germination Behavior and Early Seedling Growth in Abies pinsapo Boiss. Seeds. *Plants*, *11*(20), 1–12. https://doi.org/10.3390/plants11202715

Bravo, F., Maguire, D. A., & González-Martínez, S. C. (2017). Factors affecting cone production in Pinus pinaster Ait.: Lack of growth-reproduction trade-offs but significant effects of climate and tree and stand characteristics. *Forest Systems*, *26*(2). https://doi.org/10.5424/fs/2017262-11200

Brister, G. ., & Fry, G. (1966). Kenya Pine Timber — Structure and Strength (O . D . C .8). *East African Agricultural and Forestry Journal*, *31*(3), 343–348.

Bussmann, R. W., & Lange, S. (2000). Germination of Important East African Mountain Forest Trees. *Journal of East African Natural History*, *89*(1), 101–111. https://doi.org/10.2982/0012-8317(2000)89[101:goieam]2.0.co;2

Calama, R., & Montero, G. (2007). Cone and seed production from stone pine (Pinus pinea L.) stands in Central Range (Spain). *European Journal of Forest Research*, *126*(1), 23–35. https://doi.org/10.1007/s10342-005-0100-8

Callejas-Díaz, M., Chambel, M. R., San-Martín-Lorén, J., Gea-Izquierdo, G., Santos-Del-Blanco, L., Postma, E., & Climent, J. M. (2022). The role of maternal age, growth, and environment in shaping offspring performance in an aerial conifer seed bank. *American Journal of Botany*, *109*(3), 366–376. https://doi.org/10.1002/ajb2.1811

Calvo, L., & Nunez, M. R. (2000). Effect of high temperatures on seed germination of Pinus sylvestris and Pinus halepensis. *Forest Ecology and Management*, *131*, 183–190.

Çetin, B. (2023). The effect of altitude and closed cone (seed) age on germination in red pine (Pinus brutia Ten.). *Journal of Forestry Society of Croatia/Sumarski List Hrvatskoga Sumarskoga Drustva*, *3–4*, 129–135. https://doi.org/10.31298/sl.147.3-4.3

Chen, X., Brockway, D. G., & Guo, Q. (2021). Burstiness of Seed Production in Longleaf Pine and Chinese Torreya. *Journal of Sustainable Forestry*, *40*(2), 188–196. https://doi.org/10.1080/10549811.2020.1746914

Correia, I., Santos, L., Faria, C., Nóbrega, C., Almeida, H., & David, T. (2014). Cone to Seedling—Variation between Pinus pinaster Provenances from Contrasting Altitudes. *Forest Science*, *60*(4), 724–732. https://doi.org/10.5849/forsci.12-044

Darge, W. A. (2017). Diversity of pathogenic fungi on plantation forests of North and North-west Ethiopia. *International Journal of Phytopathology*, *06*(02), 27–34. https://www.esciencepress.net/journals/index.php/phytopath/article/viewFile/2254/1114

de la Bastide, Y. P., LeBlanc, J., Kong, L., Finston, T., May, E. M., Reich, R., Hintz, W. E., & von Aderkas, P. (2019). Fungal colonizers and seed loss in lodgepole pine orchards of British Columbia. *Botany*, *97*(1), 23–33. https://doi.org/10.1139/cjb-2018-0153

Donald, D. G. M. (1981). Dormacy control in pinus patula seed. *South African Forestry Journal*, *118*(1), 14–19. https://doi.org/10.1080/00382167.1981.9630522

Duryea, M. L. (1987). Forest Regeneration Methods : Natural Regeneration , Direct Seeding and Planting. In *Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.* https://d1wqtxts1xzle7.cloudfront.net/54392497/regen_methods-libre.pdf?1505058623=&response-content-disposition=inline%3B+filename%3DForest_Regeneration_Methods_Natural_Rege.pdf&Expires=1717092953&Signature=UzcrefhsVHZn4ZNAj8L-3yr7t5WJ6g2N9arGMnF~4IFhVtQX

Dvorak, W. B., Jordan, A. P., Romero, J. L., Hodge, G. R., & Furman, B. J. (2001). Quantifying the geographic range of Pinus patula var longipedunculata in Southern Mexico using morphologic and RAPD marker data. *The Southern African Forestry Journal*, *192*(1), 19–30. https://doi.org/10.1080/20702620.2001.10434130

Dylewski, Ł., Jagodziński, A. M., Tomaś, Ł., Myczko, Ł., & Flaherty, S. (2021). Forest stand structure and cone crop affect winter habitat use by Eurasian red squirrel (Sciurus vulgaris). *Forest Ecology and Management*, *502*(119705), 1–7. https://doi.org/10.1016/j.foreco.2021.119705

El-Kassaby, Y. A., Moss, I., Kolotelo, D., & Stoehr, M. (2008). Seed Germination:

Mathematical Representation and Parameters Extraction. *Forest Science*, *54*(2), 220–227. https://doi.org/10.1093/forestscience/54.2.220

Essl, F., Mang, T., Dullinger, S., Moser, D., & Hulme, P. E. (2011). Macroecological drivers of alien conifer naturalizations worldwide. *Ecography*, *34*(6), 1076–1084. https://doi.org/10.1111/j.1600-0587.2011.06943.x

FAO. (1985). A guide to forest seed handling with special reference to the tropics. In R. L. Willan (Ed.), *FAO FORESTRY PAPER 20/2*. FAO. http://www.fao.org/3/ad232e/AD232E06.htm

Farinha, A. C. O., Silva, J. E. P., Correia, A. C., Sousa, E. M. R., Roques, A., & Branco, M. (2018). Is Leptoglossus occidentalis entirely responsible for the high damage observed on cones and seeds of Pinus pinea? Results from a fertirrigation trial in Portugal. *Forest Ecology and Management*, *429*(July), 198–206. https://doi.org/10.1016/j.foreco.2018.07.014

Farooq, M., Usman, M., Nadeem, F., Rehman, H. U., Wahid, A., Basra, S. M. A., & Siddique, K. H. M. (2019). Seed priming in field crops: Potential benefits, adoption and challenges. *Crop and Pasture Science*, *70*(9), 731–771. https://doi.org/10.1071/CP18604

Fernando, D. D. (2014). The pine reproductive process in temperate and tropical regions. *New Forests*, *45*(3), 333–352. https://doi.org/10.1007/s11056-013-9403-7

Fulé, P. Z., Garkoti, S. C., & Semwal, R. L. (2021). Frequent burning in chir pine forests, Uttarakhand, India. *Fire Ecology*, *17*(1). https://doi.org/10.1186/s42408-021-00106-3

Ganatsas, P., Tsakaldimi, M., & Thanos, C. (2008). Seed and cone diversity and seed germination of Pinus pinea in Strofylia Site of the Natura 2000 Network. *Biodiversity and Conservation*, *17*(10), 2427–2439. https://doi.org/10.1007/s10531-008-9390-8

García-De La Cruz, Y., Hernández-Ramírez, A. M., Ramos-Prado, J. M., & Olivares-López, L. A. (2015). Fruit and seed production of two tree native species in a cloud forest from Veracruz, Mexico Producción de frutos y semillas de dos especies arbóreas nativas en un bosque mesófilo de montaña de Veracruz, México TT - Fruit and seed production of two tree. *Polibotánica*, *39*, 103–118.

Garcillán, P. P. (2010). Seed release without fire in Callitropsis guadalupensis, a serotinous cypress of a Mediterranean-climate oceanic island. *Journal of Arid Environments*, *74*(4), 512–515. https://doi.org/10.1016/j.jaridenv.2009.09.017

Ghildiyal, S. K., Sharma, C. M., & Gairola, S. (2008). Effect of temperature on cone bursting, seed extraction and germination in five provenances of Pinus roxburghii from Garhwal Himalaya in India. *Southern Forests: A Journal of Forest Science*, *70*(1), 1–5.

https://doi.org/10.2989/SOUTH.FOR.2008.70.1.1.511

Goroshkevich, S., Velisevich, S., Popov, A., Khutornoy, O., & Vasilyeva, G. (2021). 30-year cone production dynamics in Siberian stone pine (Pinus sibirica) in the southern boreal zone: a causal interpretation. *Plant Ecology and Evolution*, *154*(3), 321–331. https://doi.org/10.5091/plecevo.2021.1793

Grayson, K. J., Wittwer, R. F., & Shelton, M. G. (2002). Cone characteristics and seed quality 10years after an even-aged regeneration cut in Shortleaf Pine stands. *Proceedings of the Eleventh Biennial Southern Silvicultural Research Conference*, 310–314.

Guemri, R. El, Jaouadi, W., Mechergui, K., Alsubeie, M., Naghmouchi, S., Ouellani, S. El, & Khouja, M. L. (2019). 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. *Ekologia Bratislava*, *38*(3), 273–291. https://doi.org/10.2478/eko-2019-0021

Habrouk, A., Retana, J., & Espelta, J. M. (1999). Role of Heat Tolerance and Cone Protection of Seeds in the Response of Three Pine Species to Wildfires. *Plant Ecology*, *145*(1), 91–99.

Harlow, W. M., Côté, W. A., & Day, A. C. (1964). The Opening Mechanism of Pine Cone Scales. *Journal of Forestry*, *62*(8), 538–540. https://doi.org/10.1093/jof/62.8.538

Hauke-Kowalska, M., Borowiak, E., Barzdajn, W., Kowalkwski, W., Korzeniewicz, R., & Wawro, T. (2019). Cone and seeds variability in seed orchards and seed stands of Pinus sylvestris L. *Baltic Forestryh*, *25*(2), 187–192. https://doi.org/10.46490/vol25iss2pp187

Havelt, T., Frase, J. N., Pude, R., & Schmitz, M. (2020). Characterisation of Bioactive Ingredients in Extracts of Fresh and Dried Coniferous Trees for the Development of Sustainable Packaging Materials. *Processes*, *8*(11), 1366. https://doi.org/10.3390/pr8111366

Haymes, K. L., & Fox, G. A. (2012). Variation among individuals in cone production in pinus palustris (Pinaceae). *American Journal of Botany*, *99*(4), 640–645. https://doi.org/10.3732/ajb.1100339

He, T., Pausas, J. G., Belcher, C. M., Schwilk, D. W., & Lamont, B. B. (2012). Fire-adapted traits of Pinus arose in the fiery Cretaceous. *New Phytologist*, *194*(3), 751–759. https://doi.org/10.1111/j.1469-8137.2012.04079.x

Horstmann, M., Buchheit, H., Speck, T., & Poppinga, S. (2022). The cracking of Scots pine (Pinus sylvestris) cones. *Frontiers in Plant Science*, *13*(982756).

https://doi.org/10.3389/fpls.2022.982756

ISTA. (2011). ISTA News Bulletin : Towards automated single counts of radicle emergence to predict seed and seedling vigour. *Seed Testing International*, *142*, 44–48.

ISTA. (2023). International rules for seed testing. *International Rules for Seed Testing*, *12.*(International rules for seed testing, Zurich, Switzerland.), 345.

Iwaizumi, M. G., Matsunaga, K., Iki, T., Yamanobe, T., Hirao, T., & Watanabe, A. (2021). Geographical cline and inter-seaside difference in cone characteristics related to climatic conditions of old planted Pinus thunbergii populations throughout Japan. *Plant Species Biology*, *36*(2), 218–229. https://doi.org/10.1111/1442-1984.12309

Iwaizumi, M. G., Ohtani, M., & Takahashi, M. (2019). Geographic cline and climatic effects on cone characteristics of natural populations of Pinus densiflora throughout the Japanese archipelago. *Journal of Forest Research*, *24*(3), 187–196. https://doi.org/10.1080/13416979.2019.1603666

Iwaizumi, M. G., Ubukata, M., & Yamada, H. (2008). Within-crown cone production patterns dependent on cone productivities in Pinus densiflora: Effects of vertically differential, pollination-related, cone-growing conditions. *Botany*, *86*(6), 576–586. https://doi.org/10.1139/B08-024

Joshi, R., Punetha, P., & Singh, P. (2016). Agroforestry: A promising tool to mitigate climate change. In S. Tewari, V. . Sah, & S. . Lavania (Eds.), *Holistic Development of Agroforestry* (pp. 167–176). Jaya Publishing House. https://www.researchgate.net/profile/Manvika-Sahgal/publication/321145419_Indigenous_Fluorescent_Pseudomonas_as_Effective_Biocontrol_Agents_for_Fungal_Disease_Management_in_Dalbergia_Sissoo_Roxb_Plantations/links/5a0fcaef0f7e9bd1b2bdcee3/Indigenous-Fluore

Kaliniewicz, Z., & Tylek, P. (2019). Aspects of the process of sorting European Black Pine seeds. *Forests*, *10*(11), 1–11. https://doi.org/10.3390/f10110966

Kaliniewicz, Z., Tylek, P., Markowski, P., Anders, A., Rawa, T., & Głażewska, E. (2014). Analysis of correlations between selected physical properties and color of Scots Pine (Pinus sylvestris L.) seeds. *Technical Sciences*, *17*(3), 259–274.

Kanzler, A., Nel, A., & Ford, C. (2014). Development and commercialisation of the Pinus patula × P. tecunumanii hybrid in response to the threat of Fusarium circinatum. *New Forests*, *45*(3), 417–437. https://doi.org/10.1007/s11056-014-9412-1

Karamysheva, A., Trofimuk, L., Priyatkin, N., Arkhipov, M., Gusakova, L., Shchukina, P., Staroverov, N., & Potrakhov, N. (2020). Comparative study of the fullness of dwarf siberian pine seeds pinus pumila (Pall.) regel from places of natural growth and collected from plants introduced in northwestern russia by microfocus X-ray radiography to predict their sowing qualities. *Biological Communications*, *65*(4), 297–306. https://doi.org/10.21638/SPBU03.2020.403

Karamysheva, A., Trofimuk, L., Priyatkin, N., Arkhipov, M., & Sshukina, P. (2019). Comparative characteristics and germination of Pinus sibirica seeds collected from places of natural growth and in the St. Petersburg Botanical garden of Peter the Great. *IOP Conference Series: Earth and Environmental Science*, *316*(1). https://doi.org/10.1088/1755-1315/316/1/012017

Karrfalt, R. P., & Belcher, E. W. (1976). Evaluation of Seed Production by Cone Analysis. *24th Northeastern Forest Tree Improvement Conference*, 26–29. https://www.google.com/books?hl=en&lr=&id=bE2Ddlo6zsEC&oi=fnd&pg=PA84&dq=EVALUATION+OF+SEED+PRODUCTION+BY+CONE+ANALYSIS&ots=_9LKrxw4si&sig=76wQtE5JARZHLkEjQhKkc2oMyOk

Kaviriri, D. K., Zhang, Q., Zhang, X., Jiang, L., Zhang, J., Wang, J., Khasa, D. P., You, X., & Zhao, X. (2020). Phenotypic variability and genetic diversity in a pinus koraiensis clonal trial in northeastern china. *Genes*, *11*(6), 1–21. https://doi.org/10.3390/genes11060673

Keeley, J. E. (2012). Ecology and evolution of pine life histories. *Annals of Forest Science*, *69*(4), 445–453. https://doi.org/10.1007/s13595-012-0201-8

Keenan, R. J., Reams, G. A., Achard, F., de Freitas, J. V., Grainger, A., & Lindquist, E. (2015). Dynamics of global forest area: Results from the FAO Global Forest Resources Assessment 2015. *Forest Ecology and Management*, *352*, 9–20. https://doi.org/10.1016/j.foreco.2015.06.014

KFS. (2018). *Londiani Participatory Forest Management Plan*. CIFOR.

Khanova, E., Konovalov, V., Timeryanov, A., Isyanyulova, R., & Rafikova, D. (2020). Genetic and selection assessment of the Scots pine (Pinus sylvestris L.) in forest seed orchards. *Wood Research*, *65*(2), 283–292. https://doi.org/10.37763/wr.1336-4561/65.2.283292

Knapp, A. K., & Anderson, J. E. (1980). Effect of Heat on Germination of Seeds from Serotinous Lodgepole Pine Cones. *American Midland Naturalist*, *104*(2), 370–372. https://doi.org/10.2307/2424879

Koba, V. P., & Zhigalova, T. P. (2019). Experimental Assessment of Postfire Resilience of Pallasian Pine Seeds. *Contemporary Problems of Ecology*, *12*(7), 724–730. https://doi.org/10.1134/S1995425519070072

Köhl, M., Lasco, R., Cifuentes, M., Jonsson, Ö., Korhonen, K. T., Mundhenk, P., de Jesus Navar, J., & Stinson, G. (2015). Changes in forest production, biomass and carbon: Results from the 2015 UN FAO Global Forest Resource Assessment. *Forest Ecology and Management*, *352*, 21–34. https://doi.org/10.1016/j.foreco.2015.05.036

Kokutse, A. D., Akpené, A. D., Monteuuis, O., Akossou, A., Langbour, P., Guibal, D., Tomazello, M. F., Gbadoe, E., Chaix, G., & Kokou, K. (2016). Selection of Plus trees for genetically improved teak varieties produced in Benin and Togo. *Bois et Forets Des Tropiques*, *328*(2), 55–66. https://revues.cirad.fr/index.php/bft/article/download/id-bft-151104/31023

Kuria, N. C., Balozi, K. B., & Kipkore, W. (2019). Growth and Yield Models for Plantation-Grown Cupressus lusitanica for Central Kenya. *African Journal of Education, Science and Technology*, *5(2)*(2), 34–58. http://ajest.info/index.php/ajest/article/view/351

Lamont, B. B., Pausas, J. G., He, T., Witkowski, E. T. F., & Hanley, M. E. (2020). Fire as a Selective Agent for both Serotiny and Nonserotiny Over Space and Time. *Critical Reviews in Plant Sciences*, *39*(2), 140–172. https://doi.org/10.1080/07352689.2020.1768465

Lee, K., Lee, H., & Kang, J. W. (2021). Analysis of Cone and Seed Characteristics from Different Mating Design Strategies of Pinus densiflora for. multicaulis. *Journal of Forest and Environmental Science*, *37*(4), 309–314. https://doi.org/10.7747/JFES.2021.37.4.309 J

Li, Y., Li, X., Zhao, M. H., Pang, Z. Y., Wei, J. T., Tigabu, M., Chiang, V. L., Sederoff, H., Sederoff, R., & Zhao, X. Y. (2021). An overview of the practices and management methods for enhancing seed production in conifer plantations for commercial use. *Horticulturae*, *7*(8), 1–18. https://doi.org/10.3390/horticulturae7080252

Lixia, H., Cuicui, L. I., & Jihong, Q. (2018). Comparison of the Physicochemical Characteristics of Pinus koraiensis L. Nut Oils from Different Extraction Technologies. *Grain & Oil Science and Technology*, *1*(3), 113–118. https://doi.org/10.3724/SP.J.1447.GOST.2018.18042

Losada, J. M., Blanco-Moure, N., & Leslie, A. B. (2019). Not all ‘ pine cones ’ flex : functional trade-offs and the evolution of seed release mechanisms. *New Phytologist*, *222*, 396–407. https://doi.org/10.1111/nph.15563

Lovreglio, R., Salvatore, R., Giaquinto, P., & Leone, V. (2007). Thermal Treatments and Germination Response Over Time of Seeds From Serotinous and Non Serotinous Cones of Pinus halepensis Mill. *International Workshop MEDPINE 3: Conservation,* *Regeneration and Restoration of Mediterranean Pines and Their Ecosystems*, 155–166. http://om.ciheam.org/om/pdf/a75/00800327.pdf

MacDicken, K. G. (2015). Global Forest Resources Assessment 2015: What, why and how? *Forest Ecology and Management*, *352*, 3–8. https://doi.org/10.1016/j.foreco.2015.02.006

MacDicken, K. G., Sola, P., Hall, J. E., Sabogal, C., Tadoum, M., & de Wasseige, C. (2015). Global progress toward sustainable forest management. *Forest Ecology and Management*, *352*, 47–56. https://doi.org/10.1016/j.foreco.2015.02.005

Maia, P., & Corticeiro, S. (2022). From Cone to Seed and Seedling—Characterization of Three Portuguese Pinus pinaster Aiton Populations. *Seeds*, *1*(2), 126–135. https://doi.org/10.3390/seeds1020011

Maid, M., Kitingan, C., & Kodoh, J. (2019). Managing Planting Materials and Planting Stock Production of Tropical Tree Species. In K. C. Liew (Ed.), *Prospects and Utilization of Tropical Plantation Trees* (1st ed., pp. 29–65). CRC Press. https://www.taylorfrancis.com/chapters/edit/10.1201/9780429442773-2/managing-planting-materials-planting-stock-production-tropical-tree-species-mandy-maid-crispin-kitingan-julius-kodoh

Makhniova, S., Mohnachev, P., & Ayan, S. (2019). Seed germination and seedling growth of Scots pine in technogenically polluted soils as container media. *Environmental Monitoring and Assessment*, *191*(2). https://doi.org/10.1007/s10661-019-7249-y

Martínez-Ramos, M., Pingarroni, A., Rodríguez-Velázquez, J., Toledo-Chelala, L., Zermeño-Hernández, I., & Bongers, F. (2016). Natural forest regeneration and ecological restoration in human-modified tropical landscapes. *Biotropica*, *48*(6), 745–757. https://doi.org/10.1111/btp.12382

Marunda, C. T., Mutta, D., Gapare, W. J., & Alves, T. (2019). Situational analysis of trends in tree improvement and germplasm production and impacts on sustainable forest management in Southern Africa. *African Journal of Rural Development*, *4*(1), 1–31.

Matziris, D. (1998). Genetic variation in cone and seed characteristics in a clonal seed orchard of Aleppo pine grown in Greece. *Silvae Genetica*, *47*(1), 37–41. https://www.thuenen.de/media/institute/fg/PDF/Silvae_Genetica/1998/Vol._47_Heft_1/47_1_37.pdf

Mbinga, J. M. ., Omondi, S. F., & Onyango, A. A. (2022). Conifers: Species Diversity and Improvement Status in Kenya. In A. C. Goncalves & T. F. Fonseca (Eds.), *Conifers - Recent Advances* (pp. 101–112). IntechOpen. https://doi.org/10.5772/intechopen.101111

Mezquida, E. T., & Benkman, C. W. (2021). Forest and cone structure influence where crossbills forage in a managed Scots pine forest. *Forest Ecology and Management*, *498*(July), 119560. https://doi.org/10.1016/j.foreco.2021.119560

Miller, G. W., Stringer, J. W., & Mercker, D. C. (2007). Technical guide to crop tree release in hardwood forests. In *Proffesional Hardwood Notes* (Vol. PB1774).

Miura, S., Amacher, M., Hofer, T., San-Miguel-Ayanz, J., Ernawati, & Thackway, R. (2015). Protective functions and ecosystem services of global forests in the past quarter-century. *Forest Ecology and Management*, *352*, 35–46. https://doi.org/10.1016/j.foreco.2015.03.039

Monteleone, I., Ferrazzini, D., & Belletti, P. (2006). Effectiveness of Neutral RAPD Markers to Detect Genetic Divergence between the Subspecies uncinata and mugo of Pinus. *Silva Fennica*, *40*(3), 391–406. https://doi.org/10.14214/sf.476

Moya, D., De Las Heras, J., López-Serrano, F. R., Condes, S., & Alberdi, I. (2009). Structural patterns and biodiversity in burned and managed Aleppo pine stands. *Plant Ecology*, *200*(2), 217–228. https://doi.org/10.1007/s11258-008-9446-6

Moya, D., De las Heras, J., López-Serrano, F. R., & Leone, V. (2008). Optimal intensity and age of management in young Aleppo pine stands for post-fire resilience. *Forest Ecology and Management*, *255*(8–9), 3270–3280. https://doi.org/10.1016/j.foreco.2008.01.067

Moya, D., Espelta, J. M., Verkaik, I., López-Serrano, F., & De Las Heras, J. (2007). Tree density and site quality influence on Pinus halepensis Mill. reproductive characteristics after large fires. *Annals of Forest Science*, *64*(6), 649–656. https://doi.org/10.1051/forest:2007043

Mubai, N., Sibiya, J., Mwololo, J., Musvosvi, C., Charlie, H., Munthali, W., Kachulu, L., & Okori, P. (2020). Phenotypic correlation, path coefficient and multivariate analysis for yield and yield-associated traits in groundnut accessions. *Cogent Food and Agriculture*, *6*(1). https://doi.org/10.1080/23311932.2020.1823591

Muhtaman, D. R., Siregar, C. A., & Hopmans, P. (2000). Criteria and indicators for sustainable plantation forestry in Indonesia. In *Criteria and indicators for sustainable plantation forestry in India*. https://doi.org/10.17528/cifor/000699

Mullin, T. J., Persson, T., Abrahamsson, S., & Gull, B. A. (2019). Effects of inbreeding depression on seed production in scots pine (Pinus sylvestris). *Canadian Journal of Forest Research*, *49*(7), 854–860. https://doi.org/10.1139/cjfr-2019-0049

Mutai, O. K. (2018). *effect of seed source and post harvest handling techniques on seed quality and seed yield of Soybean*. University of Nairobi.

Mutke, S., Gordo, J., & Gil, L. (2005). Cone yield characterization of a Stone pine (Pinus pinea L.) clone bank. *Silvae Genetica*, *54*(4–5), 189–197. https://doi.org/10.1515/sg-2005-0028

Neale, D. B., & Wheeler, N. C. (2019). The conifers: Genomes, variation and evolution. In *The Conifers: Genomes, Variation and Evolution*. https://doi.org/10.1007/978-3-319-46807-5

Nel, A. (2002). *Factors influencing controlled pollination of Pinus patula* (Issue January). University of Natal, Pietermaritzburg.

Nelson, M. R., Band, L. R., Dyson, R. J., Lessinnes, T., Wells, D. M., Yang, C., Everitt, N. M., Jensen, O. E., & Wilson, Z. A. (2012). A biomechanical model of anther opening reveals the roles of dehydration and secondary thickening. *New Forests*, *196*, 1030–1037.

Neyko, I., Kolchanova, O., Monarkh, V., & Poznyakova, S. (2020). Seed productivity and variability of Scots pine (Pinus sylvestris L.) clones of Finnish origin in seed orchard in the central part of Ukraine. *Folia Forestalia Polonica, Series A*, *62*(1), 1–12. https://doi.org/10.2478/ffp-2020-0001

Ngugi, M. R., Mason, E. G., & Whyte, A. G. D. (2000). New growth models for Cupressus lusitanica and Pinus patula in Kenya. *Journal of Tropical Forest Science*, *12*(3), 524–541.

Nieto De Pascual-Pola, C., Musalem, M. Á., & Ortega-Alcalá, J. (2003). Study of some characteristics of cones and seed of Abies religiosa (HBK) Schl. et Cham. *Agrociencia*, *37*(5), 521–535.

Nöjd, P., & Isango, J. (2003). Growth periodicity of pinus patula in southern Tanzania. *Journal of Tropical Forest Science*, *15*(2), 352–358. https://www.frim.gov.my/v1/JTFSOnline/jtfs/v15n2/352-358.pdf

Novikov, A. I., Ersson, B. T., Malyshev, V. V., Petrishchev, E. P., & Ilunina, A. A. (2020). Mechanization of coniferous seeds grading in Russia: A selected literature analysis. *IOP Conference Series: Earth and Environmental Science*, *595*(1), 0–11. https://doi.org/10.1088/1755-1315/595/1/012060

Novikov, A. I., Zolnikov, V. K., & Novikova, T. P. (2021). Grading of scots pine seeds by the seed coat color: How to optimize the engineering parameters of the mobile optoelectronic device. *Inventions*, *6*(1), 1–11. https://doi.org/10.3390/inventions6010007

Omondi, J., Murithi, P., Adongo, A., Okoth, S., & Otuoma, J. (2020). Evaluating variation in seed quality attributes in Pinus patula clonal orchards using cone cluster analysis.

Journal of Forests, *7*(1), 1–8. https://doi.org/10.18488/journal.101.2020.71.1.8

Omondi, L., & Musula, P. (2011). Land Degradation of the Mau Forest Complex in Eastern Africa: A Review for Management and Restoration Planning. *Environmental Monitoring*. https://doi.org/10.5772/28532

Ongong'a, A. I. (2012). *Geospatial Analysis of Land Cover and Land Use Change in the Mau Forest Complex of Kenya*. University of Nairobi.

Onyango, A. A., Angaine, P. M., Inoti, S. K., & Owino, J. O. (2020). Patula pine (Pinus patula) cones opening under different treatments for rapid seed extraction in Londiani , Kenya. *Journal of Horticulture and Forestry*, *12*(June), 63–69. https://doi.org/10.5897/JHF2020.0633

Orwa C, A Mutua, Kindt R , Jamnadass R, S. A. (2009). *Pinus patula Pinus patula Schldl . et Cham* . (Vol. 0, pp. 1–5). Agroforestree Database:a tree reference and selection guide version 4.0.

Owino, F., & Ndinga, A. (2004). Study on forest administration and related institutional arrangements. *Lessons Learnt on Sustainable Forest Management in Africa*.

Ozel, H. B., Abo Aisha, A. E. S., Cetin, M., Sevik, H., & Zeren Cetin, I. (2021). The effects of increased exposure time to UV-B radiation on germination and seedling development of Anatolian black pine seeds. *Environmental Monitoring and Assessment*, *193*(7), 388. https://doi.org/10.1007/s10661-021-09178-9

Payn, T., Carnus, J. M., Freer-Smith, P., Kimberley, M., Kollert, W., Liu, S., Orazio, C., Rodriguez, L., Silva, L. N., & Wingfield, M. J. (2015). Changes in planted forests and future global implications. *Forest Ecology and Management*, *352*, 57–67. https://doi.org/10.1016/j.foreco.2015.06.021

Perry, D. A., & Lotan, J. E. (1977). *Opening temperatures in serotinous cones of lodgepole pine*. U.S. Department of Agriculture, Forest Service. https://books.google.com/books?hl=en&lr=&id=8wGKkTLJNboC&oi=fnd&pg=PA3&dq=These+variations+could+be+attributed+to+cone+morphology,+varying+degrees+of+asymmetry,+differences+in+scale+tension,+effect+of+environment+on+bonding+strength,+and+genetic+differenc

Perry, J. P. . (1992). *The Pines of Mexico and Central America by Jesse P . Perry*. *41*(1), 170–171. http://www.jstor.org/stable/1222523

Pinkard, L., & Keenan, R. (2012). *Plantation Forest Industry Climate Change Adaptation Handbook. October*.

Proctor, N., & Martha, M. (2016). *Common Pines of Florida*.

PROTA. (2016). *Pinus patula*. PlantUse English. https://uses.plantnet-project.org/e/index.php?title=Pinus_patula_(PROTA)&oldid=201393

PROTA. (2020). *Cupressus lusitanica*. https://uses.plantnet-project.org/en/Cupressus_lusitanica_(PROTA)

Quan, H., Pirosa, A., Yang, W., Ritchie, R. O., & Meyers, M. A. (2021). Hydration-induced reversible deformation of the pine cone. *Acta Biomaterialia*, *128*, 370–383. https://doi.org/10.1016/j.actbio.2021.04.049

Rajjou, L., & Debeaujon, I. (2008). Seed longevity: Survival and maintenance of high germination ability of dry seeds. *Comptes Rendus - Biologies*, *331*(10), 796–805. https://doi.org/10.1016/j.crvi.2008.07.021

Ramírez-Mosqueda, M. A., Iglesias-Andreu, L. G., Armas-Silva, A. A., Cruz-Gutiérrez, E. J., de la Torre-Sánchez, J. F., Leyva-Ovalle, O. R., & Galán-Páez, C. M. (2018). Effect of the thin cell layer technique in the induction of somatic embryos in Pinus patula Schl. et Cham. *Journal of Forestry Research*, *30*(4), 1535–1539. https://doi.org/10.1007/s11676-018-0663-0

Rawat, K., & Bakshi, M. (2011). Provenance variation in cone , seed and seedling characteristics in natural populations of Pinus wallichiana A . B . Jacks (Blue Pine) in India. *Annals of Forest Research*, *54*(1), 39–55.

Redmond, M. D., Davis, T. S., Ferrenberg, S., & Wion, A. P. (2019). Resource allocation trade-offs in a mast-seeding conifer: Piñon pine prioritizes reproduction over defence. *AoB PLANTS*, *11*(6), 1–11. https://doi.org/10.1093/aobpla/plz070

Reyes, O., & Casal, M. (2001). Effect of high temperatures on cone opening and on the release and viability of Pinus pinaster and P. radiata seeds in NW Spain Otilia. *Annals of Forest Science*, *64*, 219–228. https://doi.org/10.1051/forest

Reyna-González, M., Chico-romero, P. A., Ruiz-may, E., & Gonz, R. (2019). Seed Characteristics and Nutritional Composition of Pine Nut from Five Populations of P . cembroides from. *Molecules*, *24*(2057), 1–17.

Rhoades, C. C., Fegel, T. S., Hubbard, R. M., & Chambers, M. E. (2022). Limited seed viability in long-dead serotinous lodgepole pine trees in the Southern Rockies, USA. *Forest Ecology and Management*, *526*(120565). https://doi.org/10.1016/j.foreco.2022.120565

Ribeiro, S., Cerveira, A., Soares, P., & Fonseca, T. (2022). Natural Regeneration of Maritime Pine : A Review of the Influencing Factors and Proposals for Management. *Forests*, *13*(386), 1–16.

Richardson, D. M., Williams, P. A., & Hobbs, R. J. (1994). Pine Invasions in the Southern Hemisphere: Determinants of Spread and Invadability. *Journal of Biogeography*, *511-527*. https://doi.org/10.2307/2845655

Robb, L. (2020). *Seed matters 2 : Extracting and handling five-needle pine seed in Alberta*.

Romero, B., & Ganteaume, A. (2020). Does recent fire activity impact fire-related traits of Pinus halepensis Mill. and Pinus sylvestris L. in the French Mediterranean area? *Annals of Forest Science*, *77*(106), 1–19. https://doi.org/10.1007/s13595-020-01021-4

Sáenz-Romero, C., Beaulieu, J., & Rehfeldt, G. E. (2011). Altitudinal genetic variation among Pinus patula populations from Oaxaca, Mexico, in growth chambers simulating global warming temperatures. *Agrociencia*, *45*(3), 399–411.

Saenz-romero, C., & Vargas-Hernandez, J. (1994). Performance of Pinus patula genotypes selected in South Africa and growing in their native Mexican environment. *Silvae Genetica*, *43*(2–3), 73–81.

Samano, S., & Tomback, D. F. (2003). Cone opening phenology, seed dispersal, and seed predation in southwestern white pine (Pinus strobiformis) in southern Colorado. *Ecoscience*, *10*(3), 319–326. https://doi.org/10.1080/11956860.2003.11682780

Sastry, K. S., Mandal, B., Hammond, J., Scott, S. W., & Briddon, R. W. (2019). Pinus spp. In *Encyclopedia of Plant Viruses and Viroids* (No. 21; Issue 21: Pinus spp., pp. 1865–1865). Springer India. https://doi.org/10.1007/978-81-322-3912-3_1047

Savill, P. (2019). The silviculture of trees used in British forestry. In *The silviculture of trees used in British forestry*. https://doi.org/10.1079/9781786393920.0000

Schmidt, L. H. (2000). Guide to handling of tropical and subtropical forest seed: Chapter 12 - Genetic Implications of Seed Handling. In *Danida Forest Seed Centre* (Issue 2000, pp. 1–12). http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.706.5441&rep=rep1&type=pdf

Sevik, H., & Topacoglu, O. (2015). Variation and Inheritance pattern in cone and seed characteristics of Scots pine (Pinus sylvestris L.) for evaluation of genetic diversity. *Journal of Environmental Biology*, *36*(September), 1125–1130.

Shen, X., & Cho, M. J. (2021). Factors affecting seed germination and establishment of an efficient germination method in sugar pine (Pinus lambertiana Dougl.). *HortScience*, *56*(3), 299–304. https://doi.org/10.21273/HORTSCI15562-20

Shukla, A., & Kaur, A. (2018). A systematic review of traditional uses bioactive phytoconstituents of genus ehretia. *Asian Journal of Pharmaceutical and Clinical*

Research, *11*(6), 88–100. https://doi.org/10.22159/ajpcr.2018.v11i6.25178

Singh, A., Husain, M., Mir, R. A., Parrey, A. A., & Paddar, N. A. (2017). 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. *Pharmacognosy and Phytochemistry*, *6*(4), 1622–1625. https://www.phytojournal.com/archives/2017.v6.i4.1548/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

Singh, S. P., Inderjit, S. P., Singh, J. S., Majumdar, S., Moyano, J., Nuñez, M. A., & Richardson, D. M. (2018). Insights on the persistence of pines (Pinus species) in the Late Cretaceous and their increasing dominance in the Anthropocene. *Ecology and Evolution*, *8*(20), 10345–10359. https://doi.org/10.1002/ece3.4499

Sirgėdienė, M. (2019). Differences of the bog and dry site Scots pine population seedlings germination and early growth. *Acta Biologica Universitatis Daugavpiliensis*, *19*(1), 47–56. https://du.lv/wp-content/uploads/2022/02/19_1_Sirgediene.pdf

Sivacioğlu, A., & Ayan, S. (2008). Evaluation of seed production of scots pine (Pinus sylvestris L.) clonal seed orchard with cone analysis method. *African Journal of Biotechnology*, *7*(24), 4393–4399. https://doi.org/10.5897/AJB08.776

Sutton, A., Staniforth, R. J., & Tardif, J. (2002). Reproductive ecology and allometry of red pine (Pinus resinosa) at the northwestern limit of its distribution range in Manitoba, Canada. *Canadian Journal of Botany*, *80*(5), 482–493. https://doi.org/10.1139/b02-031

Tangney, R., Merritt, D. J., Fontaine, J. B., & Miller, B. P. (2019). Seed moisture content as a primary trait regulating the lethal temperature thresholds of seeds. *Journal of Ecology*, *107*(3), 1093–1105. https://doi.org/10.1111/1365-2745.13095

Tapias, R., Climent, J., Pardos, J. A., & Gil, L. (2016). Life Histories of Mediterranean Pines. *Plant Ecology.*, *171*(1), 53–68.

Tapias, R., Gil, L., Fuentes-Utrilla, P., & Pardos, J. A. (2001). 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. *Journal of Ecology*, *89*(4), 629–638. https://doi.org/10.1046/j.1365-2745.2001.00575.x

Tíscar, P. A. (2019). Geographical variation in cone traits of Pinus nigra as result of crossbill predation: Implications for assisted migration. *Forestry*, *92*(2), 188–195. https://doi.org/10.1093/forestry/cpy046

Tomback, D. F., Keane, R. E., Schoettle, A. W., Sniezko, R. A., Jenkins, M. B., Nelson, C.

R., Bower, A. D., DeMastus, C. R., Guiberson, E., Krakowski, J., Murray, M. P., Pansing, E. R., & Shamhart, J. (2022). Tamm review: Current and recommended management practices for the restoration of whitebark pine (Pinus albicaulis Engelm.), an imperiled high-elevation Western North American forest tree. *Forest Ecology and Management*, *522*, 119929. https://doi.org/10.1016/j.foreco.2021.119929

Tonguç, M., Önder, S., Gülcemal, N., & Tonguç, F. (2022). Seed, germination, and seed-reserve traits differ along an altitudinal gradient. *Journal of Forestry Research*, *33*(6), 1903–1912. https://doi.org/10.1007/s11676-022-01467-4

Tulska, E., & Aniszewska, M. (2022). Comparison of thermal and thermomechanical methods of seed extraction from larch cones based on two case studies. *International Agrophysics*, *36*(3), 197–206. https://doi.org/10.31545/intagr/150734

Tulska, E., Aniszewska, M., & Gendek, A. (2021). The kinematics of scale deflection in the course of multi-step seed extraction from European larch cones (Larix decidua mill.) taking into account their cellular structure. *Materials*, *14*(4913), 1–20. https://doi.org/10.3390/ma14174913

Tulska, E., Aniszewska, M., & Zychowicz, W. (2022). Optimization of the process of seed extraction from the Larix decidua Mill. cones including evaluation of seed quantity and quality. *Scientific Reports*, *12*(18227), 1–15. https://doi.org/10.1038/s41598-022-22942-2

Tuomainen, T. V., Himanen, K., Helenius, P., Kettunen, M. I., & Nissi, M. J. (2022). Quantitative magnetic resonance imaging of Scots pine seeds and the assessment of germination potential. *Canadian Journal of Forest Research*, *52*(5), 685–695. https://doi.org/10.1139/cjfr-2021-0273

Udval, B., & Batkhuu, N.-O. (2013). Seed and Cone Characteristics of Scots pine (Pinus sylvestris L.) from Diverse Seed Sources in Northern Mongolia. *Eurasian Journal of Forest Research*, *16*(1), 57–62.

Valera-Burgos, J., Díaz-Barradas, M. C., & Zunzunegui, M. (2012). Effects of Pinus pinea litter on seed germination and seedling performance of three Mediterranean shrub species. *Plant Growth Regulation*, *66*(3), 285–292. https://doi.org/10.1007/s10725-011-9652-4

van Zonneveld, M., Jarvis, A., Dvorak, W., Lema, G., & Leibing, C. (2009). Climate change impact predictions on Pinus patula and Pinus tecunumanii populations in Mexico and Central America. *Forest Ecology and Management*, *257*(7), 1566–1576. https://doi.org/10.1016/j.foreco.2008.12.027

Vivian, M. A., Nunes, G. C., Dobner Jr., M., Modes, K. S., Grosskopf, É. J., & Belini, U. L. (2020). Natural durability of Cupressus lusitanica, Cryptomeria japonica and Pinus taeda woods in field trial. *Floresta*, *50*(3), 1603-1612. https://doi.org/10.5380/rf.v50i3.65059

Weng, Y. H., Liu, K. J., Chen, Y. B., Li, Y., Wang, J., Meng, Q. F., Liu, K. J., Chen, Y. B., Li, Y., Wang, J., & Meng, Q. F. (2020). *Variation in cone and seed traits in a clonal seed orchard of red pine (Pinus koraiensis Sieb . et Zucc .). 7581*. https://doi.org/10.1080/02827581.2020.1725620

Willan, R. (1984). *A guide to forest seed handling with special reference to the tropics.* http://www.fao.org/3/ad232e/ad232e00.htm

Wyse, S. V., & Dickie, J. B. (2017). Predicting the global incidence of seed desiccation sensitivity. *Journal of Ecology*, *105*(4), 1082–1093. https://doi.org/10.1111/1365-2745.12725

Wyse, S. V, Brown, J. E., & Hulme, P. E. (2019). Seed release by a serotinous pine in the absence of fire: implications for invasion into temperate regions. *AoB PLANTS*, *11*(6), 1–8. https://doi.org/10.1093/aobpla/plz077

Xiang, X., Zhang, Z., & Wu, G. (2019). Effects of seed storage conditions on seed water uptake, germination and vigour in Pinus dabeshanensis , an endangered pine endemic to China . *Seed Science and Technology*, *47*(2), 229-235. https://doi.org/10.15258/sst.2019.47.2.09

Yang, Y., Ferguson, D. K., Liu, B., Mao, K. S., Gao, L. M., Zhang, S. Z., Wan, T., Rushforth, K., & Zhang, Z. X. (2022). Recent advances on phylogenomics of gymnosperms and a new classification. *Plant Diversity*, *44*(4), 340–350. https://doi.org/10.1016/j.pld.2022.05.003

Yousefpour, R., Temperli, C., Jacobsen, J. B., Thorsen, B. J., Meilby, H., Lexer, M. J., Lindner, M., Bugmann, H., Borges, J. G., Palma, J. H. N., Ray, D., Zimmermann, N. E., Delzon, S., Kremer, A., Kramer, K., Reyer, C. P. O., Lasch-Born, P., Garcia-Gonzalo, J., & Hanewinkel, M. (2017). A framework for modeling adaptive forest management and decision making under climate change. *Ecology and Society*, *22*(4), 1-25. https://doi.org/10.5751/ES-09614-220440

Zhang, Y., Hu, P., Zhong, C., Wei, Y., Meng, J., Li, Z., Pinyopusarerk, K., & Bush, D. (2020). Analyses of genetic diversity, differentiation and geographic origin of natural provenances and land races of Casuarina equisetifolia based on EST-SSR markers. *Forests*, *11*(4), 1-17. https://doi.org/10.3390/f11040432

APPENDICES

Appendix A: Data collection tools

i) Trees selection and cone collection

Tree No.	**dbh (cm)**	**Ht (m)**	**Number of cones collected**	**Remarks**

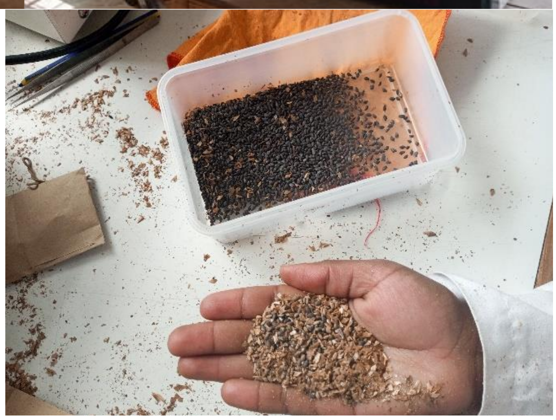
ii) Cone characteristics and grouping

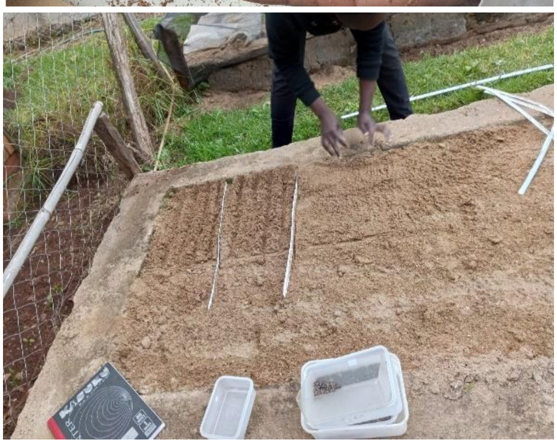
Tree no							
Cone no.	Weight (g)	Width(cm)	Category	Seed count I	Seed count II	Seed count III	Remarks

iii) Seed moisture content form recording

Seed lot	Replicate	Wt of empty petri dish with lid (g)	Wt of petri dish with lid + seed before drying (g)	Wt of Petri dish with lid + seed after drying (g)	Moisture content %	
		W1	W2	W3	(W2-W3)/(W2-W1) × 100	Mean (R I + R II)/2
	I					
	II					
	I					
	II					
	I					
	II					
	I					
	II					
	I					
	II					
	I					
	II					
	I					
	II					
	I					
	II					
	I					
	II					
	I					
	II					

Appendix B: Data collection





Appendix C: Descriptive statistics of sampled trees

	dbh	*Cone weight*	*Cone width*
Mean (*x*)	34.6	28.5	2.8
Standard Error (±)	1.21	1.20	0.04
Median	36	28.5	2.8
Mode	30	26.7	2.9
Standard Deviation	7.730262	7.690334	0.273393
Sample Variance	59.75695	59.14124	0.074744
Range	28.2	33.1	1.3
Minimum	18	9.3	2.1
Maximum	46.2	42.4	3.4
Sum	1419.3	1168.4	114.7
Count	41	41	41
Confidence Level (95.0%)	2.44	2.43	0.09

Appendix D: Variation in seed yield based on cone characteristics and phases of extraction

Treatment	L1	H1	N1	W1	L2	H2	N2	W2	L3	H3	N3	W3
Mean seed yield (*x*±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 6th hour, 6th hour to the 12th hour and the 12th hour to the 24th hour, x:group mean, se:standard error, sd:standard deviation, ci: confidence interval

Appendix E: Seed moisture content for seeds extracted at varied extraction periods

Seed Lot	W 1	W 2	W 3	H 1	H2	H3	L1	L2	L3	N1	N2	N3	W 24	H 24	L 24	N 24
mean smc	6.2 12	5.1 39	4.8 94	3.0 91	2.0 34	4.9 01	5.6 38	5.0 26	1.6 39	4.3 85	1.3 56	2.0 18	5.7 99	1.3 92	4.7 44	2.6 15

**Letters W, H, L, N denotes cone characteristics wide, heavy, light and narrow respectively. Numeric 1,2, 3 stand for extraction periods in phases 6 hours, 12 hours and 24 hours. Numeric 24 stands for 24 hours of extraction without breaks.*

Appendix F: Pairwise comparison of seed extraction performance based on cone characteristics

Descriptive statistics for seed extraction at 6 hours

	W	H	N	L	L	W	N	H	N	W	L	H
Mean	36	38	28	12	12	36	28	38	28	36	12	38
F		0.83		6.01		0.17		0.82		1.00		0.30

P(F<=f)	0.24		*0.001		0.001		0.22		0.50		0.001	
$F_{Critical}$	0.65		1.55		0.65		0.65		1.55		0.75	

Descriptive statistics for seed extraction at 12 hours

	H	N	H	W	L	N	L	W	N	W	L	H
Mean	7	5	7	11	5	5	5	11	5	11	5	7
F	1.91		0.36		1.15		0.22		0.25		0.43	
P(F<=f)	0.01		0.001		0.30		0.001		0.001		0.001	
$F_{Critical}$	1.55		0.64		1.55		0.65		0.66		0.75	

Descriptive statistics for extraction at 24 hours

	H	N	H	W	H	L	L	N	L	W	N	W
Mean	6	4	6	7	6	4	4	4	4	7	4	7
F	0.52		0.56		2.75		0.19		0.20		1.06	
P(F<=f)	0.008		0.015		*0.001		0.001		0.001		0.41	
$F_{Critical}$	0.64		0.65		1.55		0.65		0.65		1.55	

* $p \leq 0.05$ correlation is significant

Appendix G: Pairwise comparisons in cumulative seed yield based on cone characteristics

Descriptive statistics for cumulative seed extraction

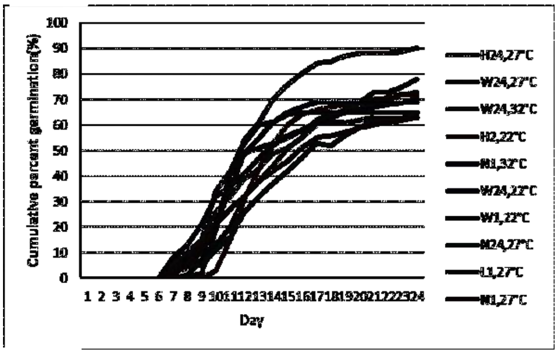
	N	W	L	N	L	H	W	H	H	N	W	L
Mean	38	56	28	38	28	50	56	50	50	38	56	28
F	0.78		0.31		0.34		1.39		0.93		4.12	
P(F<=f)	0.17		0.001		0.001		0.11		0.39		*0.001	
FCritical	0.65		0.65		0.65		1.55		0.65		1.55	

* $p \leq 0.05$ correlation is significant

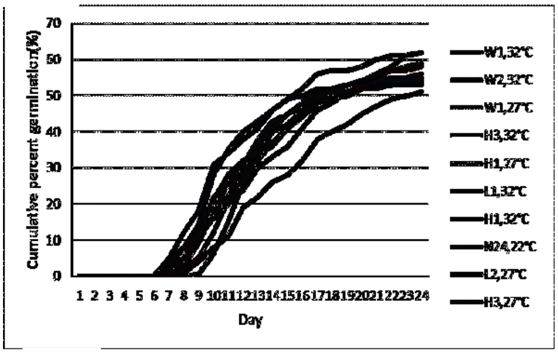
Appendix H: Examples of cone inverse deflection

Appendix I: Series of germination trends based on different seed extraction and germination conditions.

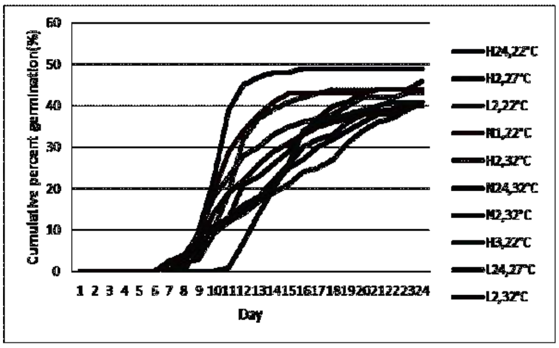
(a)



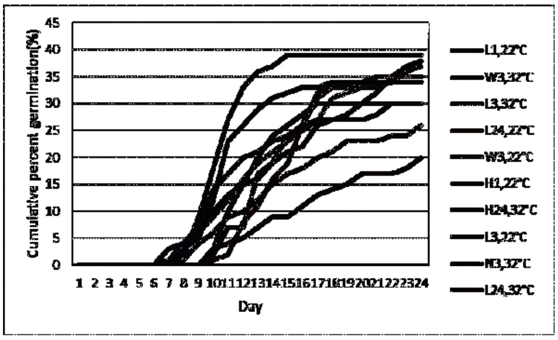
(b)



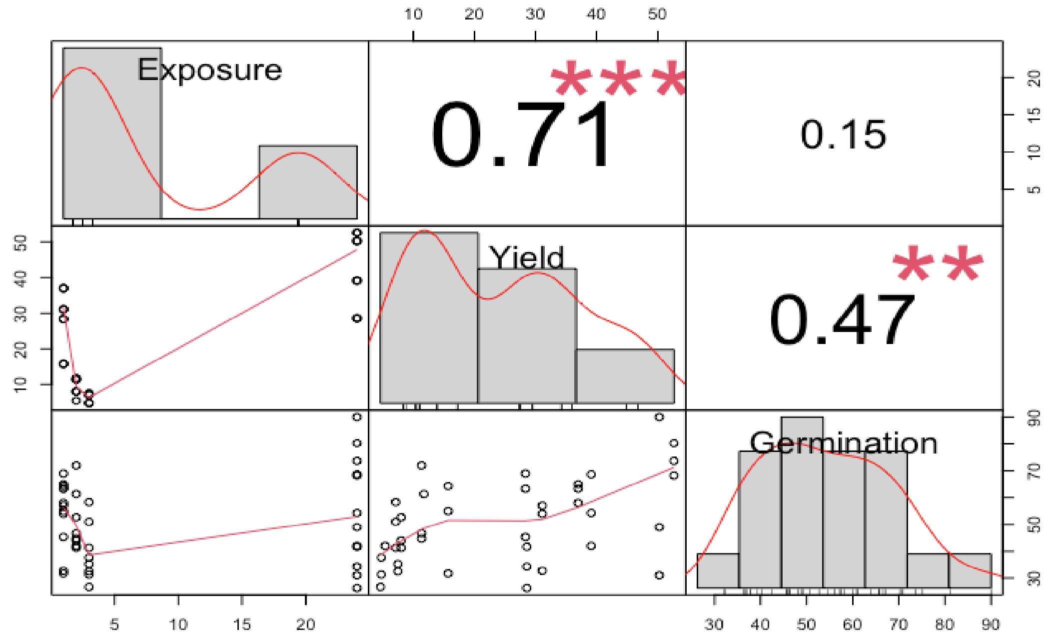
(c)



(d



Appendix J: Extraction exposure durations, seed yield, and germination correlation matrix.



Appendix K: Seedlings performance experiment in nursery conditions

Appendix L: Summary of ANOVA based on cone characteristics, extraction exposure periods and germination temperature conditions.

Source of Variation W	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Germ.temp	0.014589	2	0.007294	1.246779	0.352521	5.143253
Ext.exposure	0.288102	3	0.096034	16.41425	0.002686	4.757063

Error	0.035104	6	0.005851			
Total	0.337794	11				
Source of Variation N	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Germ.temp	0.056932	2	0.028466	4.230367	0.07143	5.143253
Ext.exposure	0.143225	3	0.047742	7.09498	0.02125	4.757063
Error	0.040374	6	0.006729			
Total	0.24053	11				
Source of Variation L	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Germ.temp	0.047241	2	0.023621	4.076726	0.076184	5.143253
Ext.exposure	0.051019	3	0.017006	2.935131	0.121283	4.757063
Error	0.034764	6	0.005794			
Total	0.133024	11				
Source of Variation H	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Germ.temp	0.047724	2	0.023862	0.618935	0.569668	5.143253
Ext.exposure	0.014232	3	0.004744	0.123054	0.943109	4.757063
Error	0.23132	6	0.038553			
Total	0.293277	11				

Appendix M: Tukey multiple comparisons of means 95% family-wise confidence level for comparison of seed yield, cone characteristics and exposure period.

One-way ANOVA: L versus T

```
Source   DF       SS     MS      F      P
T         2   1647.9  823.9  15.56  0.000
Error   174   9214.8   53.0
Total   176  10862.6
S = 7.277   R-Sq = 15.17%   R-Sq(adj) = 14.19%
                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level   N    Mean   StDev    +---------+---------+---------+---------
 6     59  11.627  10.736                          (-----*-----)
12     59   5.424   5.282      (-----*-----)
24     59   4.915   3.962    (-----*------)
                             +---------+---------+---------+---------
                           3.0       6.0       9.0      12.0
Pooled StDev = 7.277
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of T
Individual confidence level = 98.07%
T =  6 subtracted from:
T    Lower  Center   Upper  --------+---------+---------+---------+-
12  -9.368  -6.203  -3.039   (--------*--------)
24  -9.876  -6.712  -3.547  (--------*--------)
                            --------+---------+---------+---------+-
                                -7.0      -3.5       0.0       3.5
T = 12 subtracted from:
T    Lower  Center  Upper  --------+---------+---------+---------+-
24  -3.673  -0.508  2.656                   (--------*--------)
                           --------+---------+---------+---------+-
                               -7.0      -3.5       0.0       3.5
```

One-way ANOVA: H versus T

```
Source   DF     SS     MS      F      P
T         2  39478  19739  64.81  0.000
Error   174  52993    305
Total   176  92471
S = 17.45   R-Sq = 42.69%   R-Sq(adj) = 42.03%
                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level   N   Mean  StDev  ---------+---------+---------+---------+
 6     59  38.69  28.71                             (--*---)
12     59   7.81   6.81    (---*--)
24     59   6.27   6.58  (---*---)
                         ---------+---------+---------+---------+
                                 12        24        36        48
Pooled StDev = 17.45

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of T
Individual confidence level = 98.07%
T =  6 subtracted from:

T    Lower  Center   Upper  -------+---------+---------+---------+--
12  -38.47  -30.88  -23.29   (----*----)
24  -40.01  -32.42  -24.84  (----*----)
                            -------+---------+---------+---------+--
                                 -30       -15         0        15
T = 12 subtracted from:

T   Lower  Center  Upper  -------+---------+---------+---------+--
24  -9.13   -1.54   6.05                       (----*----)
                          -------+---------+---------+---------+--
                               -30       -15         0        15
```

*L=seed yield from light cones, T=Extraction period, H=Seed yield from heavy cones

One-way ANOVA: N versus T

```
Source   DF     SS     MS      F      P
T         2  21458  10729  41.37  0.000
Error   174  45121    259
Total   176  66579
S = 16.10   R-Sq = 32.23%   R-Sq(adj) = 31.45%
                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level   N   Mean  StDev  +---------+---------+---------+---------
 6     59  28.36  25.92                          (---*---)
12     59   5.39   4.92   (---*----)
24     59   4.63   9.05  (----*---)
                         +---------+---------+---------+---------
                         0        10        20        30
Pooled StDev = 16.10
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of T
Individual confidence level = 98.07%
T =  6 subtracted from:
T    Lower  Center   Upper   -+---------+---------+---------+--------
12  -29.97  -22.97  -15.96    (------*------)
24  -30.73  -23.73  -16.73   (------*------)
                             -+---------+---------+---------+--------
                            -30       -20       -10         0

T = 12 subtracted from:
T   Lower  Center  Upper   -+---------+---------+---------+--------
24  -7.76   -0.76   6.24                           (------*------)
                           -+---------+---------+---------+--------
                          -30       -20       -10         0
```

One-way ANOVA: W versus T

```
Source   DF     SS     MS      F      P
T         2  28568  14284  48.89  0.000
Error   174  50838    292
Total   176  79405
S = 17.09   R-Sq = 35.98%   R-Sq(adj) = 35.24%
                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level   N   Mean  StDev  -------+---------+---------+---------+--
 6     59  36.42  25.91                               (---*----)
12     59  11.86  11.31      (----*---)
24     59   7.59   8.78  (----*---)
                         -------+---------+---------+---------+--
                               10        20        30        40
Pooled StDev = 17.09
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of T
Individual confidence level = 98.07%

T =  6 subtracted from:
T    Lower  Center   Upper    +---------+---------+---------+---------
12  -31.99  -24.56  -17.13       (------*-----)
24  -36.26  -28.83  -21.40    (-----*-----)
                              +---------+---------+---------+---------
                            -36       -24       -12         0


T = 12 subtracted from:
T    Lower  Center  Upper    +---------+---------+---------+---------
24  -11.70   -4.27   3.16                          (-----*------)
                             +---------+---------+---------+---------
                           -36       -24       -12         0
```

*N=seed yield from narrow cones, T=Extraction period, W=Seed yield from wide cones

Appendix N: Regression equations for predicting germination

1 Regression Analysis: germ versus GT, expo by cone width

```
The regression equation is
germ = 40.7 + 0.70 GT - 0.913 expo


Predictor      Coef  SE Coef      T      P
Constant      40.69    34.28   1.19  0.269
GT            0.699    1.025   0.68  0.514
expo        -0.9128   0.2037  -4.48  0.002


S = 9.72557   R-Sq = 87.7%   R-Sq(adj) = 84.6%


Analysis of Variance

Source          DF      SS      MS      F      P
Regression       2  5393.9  2696.9  28.51  0.000
Residual Error   8   756.7    94.6
Total           10  6150.5
```

2 Regression Analysis: germ_1 versus exp, gt_1 by cone weight

```
The regression equation is
germ_1 = 35.5 - 0.238 exp + 0.500 gt_1


Predictor      Coef  SE Coef      T      P
Constant      35.50    21.15   1.68  0.128
exp         -0.2381   0.4872  -0.49  0.637
gt_1         0.5000   0.7292   0.69  0.510


S = 12.6296   R-Sq = 7.3%   R-Sq(adj) = 0.0%


Analysis of Variance

Source          DF      SS     MS     F      P
Regression       2   113.1   56.5  0.35  0.711
Residual Error   9  1435.6  159.5
Total           11  1548.7
```

*Equation 1: germ=germination performance (%), GT = germination temperature (℃), expo= extraction period (hours).

*Equation 2: germ_1= germination performance (%), exp= extraction period (hours), gt_1= germination temperature (℃)

Appendix O: List of publications

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

Asian Journal of Research in Agriculture and Forestry

8(4): 261-272, 2022; Article no.AJRAF.94823
ISSN: 2581-7418

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

Alice Adongo Onyango [a,b*], Shadrack Kinyua Inoti [a], Nelson Maara [a], James Munga Kimondo [b] and Jesse Owino [b]

[a] *Department of Natural Resources Management, Faculty of Environment and Resources Development, Egerton University, P.O. Box 536-20115, Egerton, Kenya.*
[b] *Kenya Forestry Research Institute, P.O Box 20412-00200, Nairobi, Kenya.*

Authors' contributions

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

Article Information

DOI: 10.9734/AJRAF/2022/v8i4186

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/94823

Original Research Article

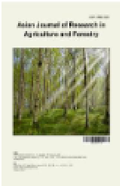
Received 19 October 2022
Accepted 21 December 2022
Published 23 December 2022

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

*Corresponding author: E-mail: adongo.alis@gmail.com;

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



Asian Journal of Research in Agriculture and Forestry

Volume 10, Issue 1, Page 61-71, 2024; Article no.AJRAF.112838
ISSN: 2581-7418

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

Alice Adongo Onyango [a,b*], Shadrack Kinyua Inoti [a], Nelson Maara [a] and James Munga Kimondo [b]

[a] *Department of Natural Resources Management, Faculty of Environment and Resources Development, Egerton University, P. O. Box 536-20115, Egerton, Kenya.*
[b] *Kenya Forestry Research Institute, P.O Box 20412-00200, Nairobi, Kenya.*

Authors' contributions

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

Article Information

DOI: 10.9734/AJRAF/2024/v10i1269

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/112838

Original Research Article

Received: 05/12/2023
Accepted: 10/02/2024
Published: 15/02/2024

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

**Corresponding author: E-mail: adongo.alis@gmail.com;*

Asian J. Res. Agric. Forestry, vol. 10, no. 1, pp. 61-71, 2024

Appendix P: Research permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 541403

Date of Issue: **01/August/2022**

RESEARCH LICENSE

This is to Certify that Ms.. Alice Adongo Onyango of Egerton University, has been licensed to conduct research in Kericho on the topic: EFFECT OF PATULA PINE (Pinus patula) CONE PHYSICAL CHARACTERISTICS AND EXTRACTION PERIOD ON SEED YIELD AND QUALITY IN LONDIANI, KENYA for the period ending : 01/August/2023.

License No: **NACOSTI/P/22/19367**

541403

Applicant Identification Number

Director General

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.