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Edited by Ana Cristina Gonçalves
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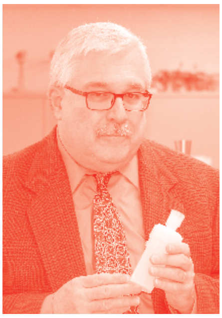

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Contributors
Ninghua Zhu, Xiaowei Yang, Zhiqiang Han, Xiao Can, Teresa Fidalgo Fonseca, Ana Cristina Gonçalves, José Lousada, Denis Charpin, Pascal Poncet, Hélène Sénéchal, Takayoshi Koike, Laiye Qu, Yannan Wang, Oxana Masyagina, Satoshi Kitaoka, Kazuhito Kita, Saki Fujita, Anatoly Prokushkin, Daniel G. George Neary, André Sandim, Maria Emília Calvão Moreira Emília Silva, Margarida Tomé, Yassine Messaoud, Joram Makaka E. M.E. Mbinga, Stephen F. Omondi, Alice A. Adongo Onyango, Wubalem Tadess

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Meet the editors

Ana Cristina Gonçalves is an Assistant Professor with Habilitation in the Department of Rural Engineering, University of Évora, Portugal, and a researcher at the Mediterranean Institute for Agriculture, Environment and Development (MED). She holds a Ph.D. in Forestry. Dr. Gonçalves has authored more than 100 publications and participated in 20 research projects. Her research is focused on silviculture and modelling in pure, mixed, and even-aged and uneven-aged stands, and forest management and planning integrated into a GIS environment.

Teresa Fidalgo Fonseca has a Ph.D. in Forestry Sciences from the University of Trás-os-Montes e Alto Douro (UTAD), Portugal. She works at UTAD where she directs courses in Dendrometry, Forest Inventory, Forest Management, and Forest Resource Modelling in graduate and post-graduate programs. She conducts research in the scientific domains of her specialty areas, being the author of scientific and technical publications of international dissemination. She is a member of the Forest Research Centre, School of Agriculture, University of Lisbon and is a modeler at the CAPSIS community. She is Deputy of Division 1 (Silviculture) of the International Union of Forest Research Organizations (IUFRO) and coordinator of the research unit 1.01.10 Ecology and silviculture of pine IUFRO.

Contents

Preface

Conifers include a wide range of species that are spread all over the world. These species have wide diversity and variable stand structures ranging from monospecific monocohort to multispecific multicohort. They produce an assortment of products and services, although the most frequent main product is timber. Due to their stand structure variability and dynamics, the characterization and modelling of the interactions between individuals as well as their growth and production comprise a set of approaches. These approaches comprise a suite of methods and techniques that make the bridge between research, and management and forest planning.

Significant issues for conifer stands are stand structure, growth, mortality, harvest, regeneration, reproductive material, diversity, yield, potential productivity, biotic and abiotic disturbances, silvicultural practices, system sustainability, and perpetuity. This book provides a comprehensive overview of the current state of the art in this field. It examines the three pillars of sustainability – environmental, social, and economic – presenting reviews and research studies in the following areas: silviculture, modelling, forest planning, diversity, forest management, abiotic factors, and conifer pollen allergies.

This book is a collection of contributions from several fields and perspectives on the characterization, modelling, management, and diversity of conifer stands and forests, with wide geographical coverage. It includes nine chapters:

Chapter 1: “Maritime Pine, Its Biological and Silvicultural Traits for the Basis of Natural Resources: An Overview”

Chapter 2: “Complexity of Regeneration Dynamic at the Ecocline between Mixedwood and Coniferous Domains of the Southernmost Boreal Zone in Eastern North America”

Chapter 3: “Research Progress on Iron-Heart *Cunninghamia lanceolate*”

Chapter 4: “Conifers: Species Diversity and Improvement Status in Kenya”

Chapter 5: “Larch: A Promising Deciduous Conifer as an Eco-Environmental Resource”

Chapter 6: “*Pinus patula* Plantations in Africa: An Overview of Its Silvicultural Traits and Use under SDG”

Chapter 7: “Management of *Pinus pinaster* Aiton for Wood and Resin Production: A Technical-Financial Feasibility Analysis”

Chapter 8: “Recent Megafires Provide a Tipping Point for Desertification of Conifer Ecosystems”

Chapter 9: “Respiratory Allergy to Conifers”

Editing this book was very rewarding and we hope readers, including researchers and students, will find it to be a valuable reference.

Ana Cristina Gonçalves
Department of Rural Engineering,
School of Sciences and Technology,
MED - Mediterranean Institute for Agriculture,
Environment and Development,
Institute of Research and Advanced Information (IIFA),
University of Évora,
Évora, Portugal

Teresa Fidalgo Fonseca
University of Trás-os-Montes e Alto Douro,
Vila Real, Portugal

Forest Research Centre,
School of Agriculture,
University of Lisbon,
Lisbon, Portugal

Chapter 1

Maritime Pine, Its Biological and Silvicultural Traits for the Basis of Natural Resources: An Overview

Teresa Fidalgo Fonseca, Ana Cristina Gonçalves and José Lousada

Abstract

Maritime pine (*Pinus pinaster* Aiton) is a forest tree species with a high representation in southwestern European countries, in particular Portugal, Spain, and France. The species traits and their flexibility and plasticity are of importance both for timber and to the sustainability of the forest systems. Extensive research has been made on the maritime pine systems and productions. The aim of this study is to review the state-of-the art on the knowledge of the species, their forest systems, and their productions, to identify vulnerabilities and to summarize tools to help its management. The specific objectives of this review are: i) characterizing maritime pine, its distribution, genetic material and provenances, the biotic and abiotic disturbances, the diversity and sustainability of its forest systems; (ii) its management, encompassing the silvicultural systems and practices; (iii) to list existing growth models, simulators and decision support systems; and (iv) present information on wood technology, including sylvotechnology, wood properties, and their use.

Keywords: species traits, distribution, silviculture, models, wood technology

1. Introduction

Maritime pine (*Pinus pinaster* Aiton) is a conifer with a large area of distribution and of particular value, namely in terms of provisioning, regulating, and supporting ecosystem services. In Europe, its main distribution occurs in the Southwest Atlantic region (Portugal, Spain, and France), and to a lesser extent in other regions of Mediterranean influence. It has also been successfully introduced to other continents. One major benefit of the maritime pine forests, inherently associated with its expansion, is wood production and the supply of timber. The species plasticity and rusticity associated with its many functions, from production to protection, is linked to its wood quality and yields, make it a specie of primordial importance in several countries. Currently, it is prone to a suite of abiotic and biotic disturbances (e.g., fire, drought, pests, and diseases), which can act simultaneously or not. The forest system and production sustainability have to be thought holistically, with the selection of the better-suited management systems and sites to promote optimized yields and wood quality. The aim of this review is to provide information on the state of the technical knowledge of maritime pine and its forest systems.

The objectives are fourfold: (i) distribution and ecology of maritime pine (Section 2); (ii) silviculture (Section 3); (iii) models, simulators, and decision support systems (Section 4), and (iv) wood technology (Section 5).

2. Distribution and ecology of *P. pinaster*

Maritime pine (*P. pinaster* Aiton) is an evergreen conifer species belonging to *Pinaceae* and *Pinus* genera. It is a plastic specie characterized by its fast growth, shade intolerance, and being rustic ([1, 2] and references therein). Its area of distribution ranges from Portugal to Greece and from Morocco to Tunisia, whether as continuous ancient or recent areas (**Figure 1**, [3]). The specie is reported as native in France, Italy, Spain, Morocco, and Portugal [4]. It can be found outside its natural range in Australia, New Zealand, South Africa, Chile, Argentina or Uruguay [5], Turkey, the Balkans, United Kingdom, and Belgium (**Figure 1**, [3]). Its distribution is probably associated with the species traits' plasticity and wood quality. The specie's prolific seed production, wind-dispersed seed, and rapid growth rate, support the qualification of the species as an aggressive colonizer in some of the countries where it was introduced [4].

Maritime pine develops for a range of mean annual temperature between 13 and 15°C, and 8 to 10° C in the colder months, mean annual precipitation larger than 800 mm (100 mm in the dry season), altitudes up to 800 m. It has low sensitivity to autumn and winter frosts, but high to spring ones, and has a high sensitivity to snow. It prefers soils of light texture, with good drainage and with a depth larger than 30 cm, where root systems develop better but do not tolerate, calcareous, saline, hydromorphic, and compacted soils. Its ability to grow in shallow and

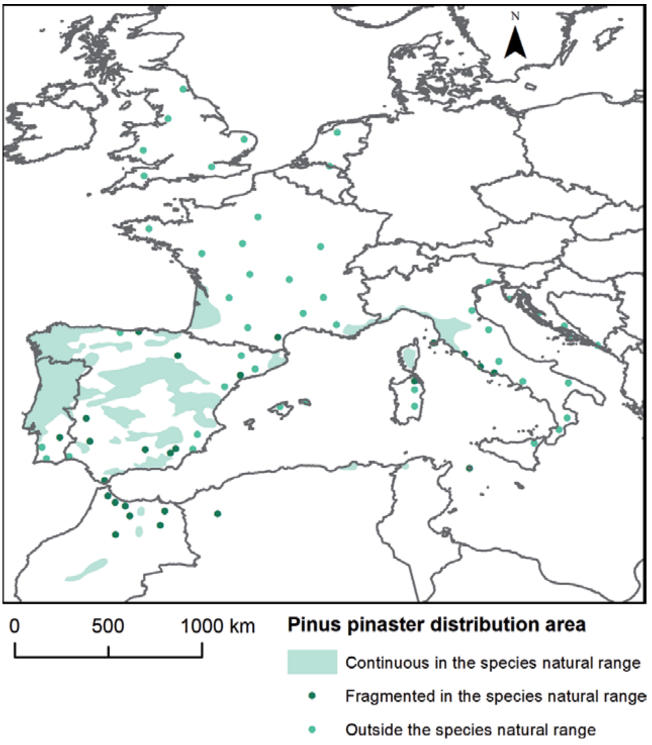

Figure 1.
Area of distribution of maritime pine (source: Caudullo et al. [3]).

nutrient-poor sites is due to not being very demanding regarding mineral nutrition and by establishing ectomycorrhizal associations that improve its ability to uptake nutrients in soils with pH less or equal to 5 [1, 2, 6–8]. The root system consists of superficial roots, which ensure the stability of the tree and support the fine roots, responsible for the absorption of water and nutrients, and deep roots, which ensure the attachment to the soil and the tree's access to water from deeper groundwater levels [6]. It provides good anchorage regardless of soil water content, except when in full saturation in sandy soils [9]. Nonetheless, the lower the nutrients' availability the lower the potential growth of the trees [10, 11]. It reaches 30–40 m in height [12], its longevity is between 80 and 300 years [1, 2] and it is shade intolerant [2]. It resists well the summer water deficits, characteristic of the Mediterranean region, as due to the high sensitivity of the stomata to water deficit it is able to maintain tissue hydration at adequate levels [13, 14]. Its imminently pioneering character is notorious in the success of its use in the fixation of coastal dunes formed by sands poor in organic matter, minerals, and water retention capacity [2].

In France, maritime pine occupies an area of 1015 thousand hectares, with the Landes having the largest monospecific area. While it represents 5% of the metropolitan French forested area, it is the most harvested species with 6.7 Mm^3/year of removals [15]. It is also widely distributed in northwest Spain, in the Autonomous Communities of Galicia and Asturias and the province of León, and is the most important coniferous tree species in terms of both surface cover, with an area of 433,754 ha, and wood production [16, 17] with a volume harvested in 2017 of 3.4 Mm^3 [16]. In Portugal mainland, its distribution extends along a coastal strip of low altitude from North to South as well as in the inner North and Central regions, up to an altitude of 700–900 m mainly under Atlantic climatic influence, and mostly in the Southwest to North aspects. It is the most represented conifer species in northern and central Portugal, occupying an area of 713.3 thousand hectares and a growing stock of 67 Mm^3 [18]. Wood availability is estimated at 1.8 Mm^3, in 2018, with a consumption of 4.2 Mm^3 [19]. Typical stands are shown in **Figures 2**–**4**.

Figure 2.
A mature stand of Pinus pinaster *(Mata Nacional de Leiria, Portugal).*

Figure 3.
Natural regeneration of Pinus pinaster *after clearcutting (Mata Nacional de Leiria, Portugal).*

Figure 4.
Adult stand of Pinus pinaster *(Vale do Tâmega, Portugal).*

The importance of maritime pine is not confined to its area, but it is also related to its economic returns and goods and services its stands and forests provide. Maritime pine major products (wood and resin) have a wide variety of uses, involving a complex forestry-industrial sector and integrating, in addition to the set associated with the transformation of wood, a range of enterprises processing non-woody forest raw materials, with emphasis on resinous products. Its contribution to the national economies is relevant. For example, in Portugal, this sector has 8516 companies and is responsible for 57,843 employees (representing 88% of industrial companies and 81% of employment in the Forestry Sector) and generate 1225 € million of Gross Value Added, €4348 million of Turnover, and €1876 million of exports (3.1% of national exports of goods) [19, 20].

In Portugal, it is the main wood-producing species for general purposes, which, in addition to a medium wood density, combines good strength characteristics and easy working. According to [20], of the 4.5 million m^3 of *P. pinaster* wood consumed in 2019 in Portugal, 1.82 million m^3 corresponded to timber wood, 1.07 million m^3 to pellets, 0.68 million m^3 to wood panels, 0.56 million m^3 for pulp and paper, 0.20 million m^3 for biomass, and 0.15 million m^3 for poles, pilings, posts, and sleepers. In addition, it has also to be highlighted the production of resin extracted from this species, which in the last 8 years has ranged from 6000 to 8000 t per year [20].

The importance of this sector goes far beyond the purely economic aspects, as its stands are essential for the populations life quality, with a direct impact on the quality of air, soil, and water and, in general terms, in the surrounding ecosystem. For example, *P. pinaster* forests constitute the largest carbon reservoir in the Portuguese forest (90.3 Gg CO_2) and also the most carbon stored per hectare (119.4 t CO_2/ha) [20].

Maritime pine stands, due to its low crown cover, result of its shade intolerance, enable the development of an herbaceous and shrub understorey. This understory encompasses a suite of species resulting in moderate to high species richness. Also, it serves as shelter and reproduction spots for several bird, mammal, and reptile species [21]. Diversity is also enhanced by the different stand structures, from pure even-aged to mixed uneven-aged [22–24].

The sustainability of the pine stands and their productions are dependent on their resilience to disturbances, which include type, intensity, and frequency. Silvicultural practices are disturbances of low intensity and high frequency, with the aim of promoting growth. In general, its effects promote the system sustainability. Inversely, high intensity and low-frequency disturbances, such as fires or storms, may endanger the system sustainability [25]. Maritime pine stands are prone to fires, especially when a well-developed understory promotes the continuity of the vertical profile of the stand. The effects of forest fires on forest stands in general, and on maritime pine in particular, are twofold: the destruction of the stand and effects on soil. The resilience of the stand is linked to the regeneration which in turn is associated with the intensity of destruction (total or partial), type of regeneration (sexual or asexual reproduction), and the availability of seeds (whether in the soil or in the tree crowns). Maritime pine regenerates by seed (it is not able to sprout) and as long as seed is available, stand regeneration occurs [26]. It is well known the effect of vegetation on soil conservation and reduction of erosion risk, which is especially relevant in climates subject to high-intensity rainfalls, such as the Mediterranean climate. Also, vegetation, especially the arboreal, gives a primordial contribution to the maintenance and improvement of the soil's physical, chemical, and biological properties, thus contributing to maintain and improve site quality ([27] and references therein). Maritime pine stands are frequently in sites of low quality, many times in steep slopes areas with high-intensity rainfalls [28, 29]. Thus, its sustainability can be enhanced by disturbances of low intensity and high frequency, such as the silvicultural practices (thinning and pruning) that can prevent those of high intensity and low frequency, such as fires. Maritime pine is also vulnerable to wind damage [30]. Extreme wind events associated with severe extratropical cyclones (storms) have caused extensive damage in Europe. In France, Nouvelle-Aquitaine region, the damage of Martin and Klaus storms affected predominately maritime pine (37 million m^3), which correspond to 15% and 32% of the maritime pine standing volume in the region in the former and latter storm, respectively [31]. The uprooting of trees and stem breakages have been reported for the species in Portugal [32–34], which may result from soil characteristics and individual tree social status, and the critical turning point at the base of the stem was correlated to tree size and particularly to stem weight or volume [35].

Among the biotic agents affecting the species, the pine processionary moth, *Thaumetopoea pityocampa* (*Lepidoptera*, *Thaumetopoeidae*) is referred to as the most serious pest in the Mediterranean region [4, 36]. The species is susceptible to *Bursaphelencus xylophilus*, the nematode that causes the pine wilt disease [4, 37], and to root rot pathogen *Heterobasidion annosum* [38]. Bark beetles (*Ips sexdentatus*, *Orthotomicus erosus*, *Tomicus piniperda* and *T. destruens*) are also referred to as the main biotic agents causing economic losses to the species [37].

The maritime pine stands sustainability is also linked with climatic change. The increase in temperature and decrease of precipitation may result in a trend to its northwards distribution [28, 29]. Also, it seems that there will be a trend towards a longer dry season in the Mediterranean. One way to mitigate its effects is by reducing density through thinning in maritime pine stands and/or with mixed stands [39, 40] of maritime pine with other conifer or broadleaved species (Section 3.1).

3. Silviculture of maritime pine (*P. pinaster*)

3.1 Forest systems

Maritime pine is managed in high-forest stands [2] (see **Figures 2** and **4**). The structure is most frequently even-aged, whether from natural [41–44] (**Figure 3**) or artificial [2, 40, 45, 46] regeneration. Traditionally, maritime pine is managed in pure stands. The preference for even-aged stands is related to easier management, promotion of wood quantity and quality [2, 40, 47–51], and disturbances, mainly fire or harvest events that usually result in one regeneration cohort shortly after disturbance, if seed is available [41–44].

The uneven-aged structure is less frequent [22, 24, 42] probably due to the specie traits. Uneven-aged stands are more frequently developed with shade-tolerant species. Yet, uneven-aged stands have been successfully developed with shade-intolerant species with few cohorts (1 to 4) [52–54]. Several studies compare and discuss even and uneven-aged stands of maritime pine [55–57]. Uneven-aged stands of maritime pine are frequently originated from natural regeneration, whether as pure [22, 42, 47, 58] or mixed stands [23, 24, 59, 60].

The advantages of mixed stands in what concerns the stands' sustainability while attaining similar or better yields than pure stands [52] enhanced the spread of maritime pine mixed stands. Examples are: *P. pinaster* and *P. sylvestris* [46, 50, 60]; *P. pinaster* and *P. pinea*, *P. sylvestris*, *P. halepensis* or *P. nigra* [39]; *P. pinaster* and *Quercus pyrenaica* [61]; *P. pinaster* and *P. radiata* [45]; *P. pinaster*, *Castanea sativa* and *Quercus robur* [23, 24]; and *P. pinaster* and *Eucalyptus* spp. [62]. While some mixed stands are originated from plantations [45] others are the result of natural regeneration [24, 42, 62]. Overall, mixed maritime pine stands have higher diversity [24, 50]; soil fertility is enhanced [50]; have a higher water holding capacity [63], and higher yields [60].

The development of maritime pine is determined by four broad factors; water availability, aerial growing space availability, tree "social" status (based on tree's height relative to surrounding trees), and silviculture practices. Maritime pine stands in the Mediterranean climate are constrained by the available water.

Several references [59, 61, 64–69] indicate that growth occurs mainly in spring and autumn as a result of precipitation [67]. A study on the effect of precipitation on water uptake in maritime pine, stresses the effects of the temporal variability of rainfall and site on the water availability [67]. As the water absorption by maritime pine individuals does not occur immediately after the rainfall but has some delay in time [67, 68], it is better explained by a set of events of rainfall [67]. Also, summer precipitation (from May to September) seems to have low contribution to the absorption of water for two reasons: the precipitation amount is low and it is partially lost through evaporation. In mixed stands of *P. pinaste*r and *Quercus pyrenaica*, spring growth of maritime pine is promoted in the early spring because leaf area is available prior to the oak's [59, 61] and due to the maritime pine root system, which is able to develop in depth thus exploring a large volume of soil [70, 71]. Also, when under water deficit, maritime pine ceases growth both in spring and fall [59, 61, 64]. In fall, trees are able to grow if water is larger than what is needed for the rehydration [59, 64]. The geographic origin along with the climate influences the tree growth reaction to drought, with higher growth under Atlantic climates than under the Mediterranean ones, which is related to the xeric climate adaptation of the species [72].

All species have, to a lower or wider extent, plasticity which enables individuals to adapt to the available growing space, by maintaining or increasing light intersection, water and nutrients absorption, and reducing competition. Species plasticity results in the variation of tree allometry, which enables the maintenance of growth. Crown plasticity can be the result of stand structure and/or climatic conditions [52]. For maritime pine individuals the increase in density results in the reduction of crown size due to crowding, when individuals do not have enough aerial growing space, or when branch abrasion occurs. These phenomena constrain the lateral growth of the crown and being maritime pine shade-intolerant, the lower crown under shade dies, resulting in the regression of the crown [39, 73]. Drought also affects crown allometry. In sites prone to drought its crown tends to have a large volume. The larger crown volume can be explained by the stands' low density, being trees in free growth thus expressing the growing habits characteristic of the specie; and as the main limiting factor is water; it is expected that belowground competition is higher than that above ground. As a consequence, the crown competition and the variability in its allometry are weaker on dry sites and stronger on humid ones [39]. Likewise, the increase of aridity decreases productivity both in pure and mixed stands, whether for volume [73] or for biomass [74].

Individual tree social status influences tree allometry and growth. In pure stands, the individuals in the lower social status (dominated) have lower sizes and growth rates, due mainly to the lower availability of growing space, light in particular. In mixed stands of *P. pinaster* and *P. sylvestris*, it was found a negative effect on dominated maritime pine individuals, probably due to the shade casted to those individuals. Inversely, in the admixtures of *P. pinea* and *P. pinaster*, and *P. nigra* and *P. pinaster,* the effects on the dominated trees were positive, which can be attributed to the different crown architecture of the species [39]. In *P. pinaster* and *P. sylvestris*, pure and mixed stands [60], maritime pine crowns in mixtures had smaller volumes (related to the specie shade intolerance), than in pure stands, and high competition for light was also found. Inversely, *P. sylvestris* tends to keep its lower branches (as it is more tolerant to shade). Also, maritime pine tends to increase its height growth to enable the individuals to reach the upper canopy layer, and, thus to reach sunlight. This results in the ascension of its crowns, which is enhanced by the crown regression (i.e., the death of the shaded lower branches) and by the development of branches with steep angles in relation to the stem. The different behavior of the two species might promote the stand vertical stratification and the optimization of the available canopy space [60]. The former and the higher capacity to hold water off the mixed stands [63] may, at least partially, explain the increase in productivity [60], stocking, and total organic carbon [75] found in mixed stands when compared to pure maritime pine stands.

Defoliation in maritime pine individuals results in the reduction of growth, of −0.9% of increment in basal area per 1% reduction of leaf area. For 15–30% of defoliation, the reduction of growth is considerable [49]. In a drought study, Rodriguez-Vallejo [40] observed that leaf area reduction due to drought resulted in the reduction of tree growth and that in natural stands was lower than in plantations. The reduction of growth due to leaf area reduction is related to the decrease of transpiration, hydraulic conductivity, and increase in xylem embolisms as well as competition for water. Thinning reducing competition may mitigate drought impacts on tree vigor and growth in maritime pine plantations [40].

Differences in tree allometry can also be assessed based on the configuration of the tree stem profile and have a direct influence on stem volume. Calçada-Duarte [76] points to a large number of geometric volume shapes for the species, varying from paraboloid to a solid of intermediate features of cone and neiloid (stem form with high tapering), which can result in stem volume differences greater than 25% for trees with equal values of diameter at breast height and total tree height.

3.2 Silvicultural practices

The most frequent silvicultural practices in maritime pine stands are thinning and pruning. Thinning is used to regulate stand density. The goal is to maintain the best trees, that will reach the end of the production cycle and remove those that have lower growth rates (dominated), less desired stem shapes, or are dead or diseased [77] while providing intermediate economic revenues. The most frequent thinning method is from below (e. g., [2, 47]). This method is used because it is suited for shade-intolerant species and for sites with periodical drought season [77], which is the case of the maritime pine stands in the Mediterranean basin with an annual summer drought period. Thinning is of importance in these stands due to its effects on tree and stand growth; wood quality and quantity, especially when associated with pruning; and system sustainability, particularly to disturbances such as fire and drought. Due to its shade intolerance, their release should be done early in stand development [2, 78].

The thinning intensity can be based on empirical rules or defined by objective criteria, being usual to use of Wilson's spacing factor [79] or Hart-Becking spacing index (H-B), widely used in France for coniferous trees (e.g., [80]), and Stand Density Index [81], the latter based on the self-thinning theory law. Density regulation based on SDI relies on the assumption that in monospecific even-aged populations of trees experiencing complete crown closure, mortality is density-dependent. The natural trajectory of the number of trees per tree size was defined by Luis and Fonseca [82] and revised by Enes et al. [44]. The use of relative values of SDI is suitable for management purposes, as it provides information on the appropriate number of living trees for given tree size, according to the management aims (e.g., optimum growth-density interval, maximization of stand volume, or maximization of mean tree size).

Arellano-Pérez [47] in maritime pine pure even-aged stands, used thinning from below with two intensities, light (removal of 20% of basal area) and heavy (removal of 40% of basal area), and compared them with unthinned plots. The authors observed that growth in diameter was the largest in the heavy thinning plots while total and crown base were similar in all treatments. Six years after thinning basal area was the largest in unthinned plots. The fuel load was lower in thinned plots, but that of the understorey had a slight increase in the thinned plots. Thinning reduces the probability of active crown fire probability but increases passive one. Overall, according to Arellano-Pérez [47] thinning did not affect fire severity and reduced potential fire risk. The effect of density on maritime pine growth is related to competition for growing space. The higher the density attained, the lower the growth, especially in diameter [58, 83]. Stands with high density are exposed to longer periods of hydric stress, especially during the drier months. Inversely, in low density stands, individual trees develop larger (deeper and wider) root systems, thus reaching water stored in the lower soil layers [83].

Nunes et al. [84] in a thinning from below experiment in maritime pine pure even-aged stands with intensity ranging from light to heavy, highlighted its importance in diameter growth while height growth was not affected. Another study in a mixed stand of *P. pinaster* and *Quercus pyrenaica* [59] observed the highest radial increments with heavy thinning intensity. The difference between treatments corresponded to the spring growth (earlywood) and was constrained especially by water availability, *i.e.*, under drought, there was a reduction of radial growth. Inversely, the autumn radial growth (latewood) does not seem to be affected by thinning, probably because it is highly dependent on the precipitation amount [59].

Pruning is a silvicultural practice frequently associated with thinning. Its main goal is to form a knot-free wood stem as high as possible, the reduction of the knotty stem core (both in number and size) and to stop juvenile wood growth [2, 85]. Pruning is recommended for two reasons: to reduce the knots number and size, which is one of the most derogatory wood features when used for nobler applications (e.g., veneer, plywood, structural elements, and furniture), both in the wood appearance characteristics and their mechanical resistance [86, 87]; and the removal of the less photosynthetically efficient branches (frequently the lower), enabling an increase of the carbohydrate availability, thus increasing growth [88, 89]. Yet, pruning removes both dead and live branches, the latter reducing also leaf area, which may also reduce photosynthesis and thus growth [90]. Hevia et al. [45] evaluated the effect of light (12–15% crown removal) and heavy (29–37% crown removal) pruning in young (7–11 years old) pure even-aged stands of maritime pine, and compared the results with unpruned trees. The higher the pruning intensity is, the greater will be the reduction of diameter growth, while lower effects were detected for height growth. Similar results were attained by Courdier et al. [91]. The effects of pruning intensity are related to species traits, namely the architecture of the crown, leaf surface area, photosynthesis, shade tolerance, and growth rates; but also, to edaphic and climatic site characteristics [45]. Hevia et al. [45] observed that the increase of growth post pruning was related to site index, relative spacing index, age, and tree diameter, as well as stand structure prior to pruning. The authors mentioned that the better the site, the older the trees, and the larger the diameter, the higher the growth in diameter and height. The post pruning growth seems to be also linked to the reserves in carbohydrates; the larger the reserves the higher the growth ([45] and references therein).

3.3 Stand regeneration

The regeneration of a stand is linked to its forest system. Clear cutting is associated mainly with artificial regeneration while clear-cutting with standards, clear-cutting by strips and/or patches, and shelterwood systems are frequently linked to natural regeneration [92, 93]. The most frequently used regeneration systems in maritime pine stands are clear-cutting, clear-cutting with standards, and clear-cutting by strips [2, 46].

Natural regeneration encompasses a set of sequential steps, namely seed production, seed dispersal, germination, and seedling establishment. Maritime pine trees are self-fertile. Wind pollination helps to spread their pollen grains from the male sexual organs (cone) to the female ones. Flowering, fruiting, and seed production are dependent on the tree development stage, stand density, and climate. Maritime pine individuals start to fruit at about 10–15 years old, with a periodicity of masting cycles of 3–5 years [2]. Trees with larger dimensions produce higher cone yields. Trees with larger dimensions tend to be in the upper layer of the canopy, are more vigorous and the light crow area is larger, all of which contribute to the increase of cone production [43, 94]. The reduction of density through thinning, reducing competition, and promoting the increase of crown area, especially the outer one where flowering and fruiting occur, increases fruit yield [43, 94].

Cone full development needs 2 years to be achieved [2] and climate, especially precipitation, determines the number of mature cones per year [43, 95]. For maritime pines stands the seed production per year is enough to regenerate the stands, in spite of its interannual variability [94, 96]. Its seeds are mainly wind dispersed; thus, wind direction and intensity are key factors in its dispersal, which occurs in the summer, from June to August [2]. The mean and the maximum dispersal distances of the seed are circa 14–25 m and 54 m, respectively [97].

Germination is related to seed germination rate and predation both before and after dispersal. Maritime pine germination occurs either in spring or autumn [2] and it is dependent on nutrient availability as the seed have few reserves; water, the increase in water stress reduces the germination and survival rates; and light environment, as germination and early development of seedlings is promoted by semi-shade environments that reduce light intensity and soil temperature, and increase soil moisture [78, 94, 98]. Guignabert et al. [94] mentioned that drought in summer was the primary cause of death in seedlings, mainly due to the increase of the deficit in vapor pressure and transpiration of seedlings. Partial cutting reduced water stress, thus promoting seedling survival [94] and a crown cover of about 32% had higher germination and survival rate of seedlings when compared with a crown cover of circa 5% [98].

Guignabert et al. [94] comparing seedlings with partial cutting clearcutting observed that seed production and dispersal were not limiting factors to regeneration. Inversely, the storage and conservation of seed in the seedbank constrained germination because of the high predation after dispersal; harvest residues and litter layer did not allow seeds to reach the soil; the capacity of germination of seeds was lower on clearcutting, and the germination rate was high in the first year after seed rain (previous year to harvest) and drastically reduced in the two following years.

Seed predation is a primordial factor in maritime pine regeneration. Predation before dispersal occurs when fruits are in the maturation early stages, while predation after dispersal takes place in the ground prior to germination, mainly by birds and insects. Post dispersal seed predation happens mostly in autumn and winter and depends on seed and predators' number, frequently having a trend towards a high spatial and temporal variability [99]. Ruano et al. [96] observed that predation reduced seed of maritime pine from 400,000–500,000 seeds/ha to 10,000 seeds/ha, and that the seed predation rate increased with the decrease of quantity of seed.

3.4 Stand structure dynamics

Stand structure dynamics is determined by the initial species composition and proportions and structure. The differences in stand structure, even if they are small, may be, and many times are, enlarged in time [25]. These differences are visible both in the estimates of the stand variables and their precision and accuracy, which reinforces the need to develop flexible models that accommodate the variability of growth patterns and interactions between individuals for the variability in stand structure [52]. Alegria [42] and Alegria and Tomé [22] developed growth models for maritime pine uneven-aged stands. In both studies, the authors referred that the existing models (developed for even-aged stands) are not able to accommodate the differences in structure, and the new models outperformed the existing ones. Gómez-García [100] developed height-diameter functions for *P. pinaster* mentioning that mixed models were able to accommodate the variability in tree allometry as well as the limitations on the available data. Riofrío et al. [46] developed height-diameter functions for *P. pinaster* and *P. sylvestris*, pure and mixed even-aged stands. The model was able to accommodate the different patterns between trees and species, and account for the different species traits, allometry, and interactions. Also, the authors reported that these models had better performance than those existing for pure even-aged stands.

In maritime pine even-aged stands, rotation can be defined for a target age or diameter. Rotation age varies between 35 and 45 years [2], though longer rotations have been used, for example in coastal dunes of Mata Nacional de Leiria (see **Figure 2**), of 70 years for timber and 100–140 years for protection [101]. The target diameter is defined according to the use of wood with 7–14 cm of diameter at breast height for panels and pulp; 14–20 cm for timber and > 35 cm for veneer wood and large dimension timber [2]. **Figure 5** presents *P. pinaster* wood logs, after logging.

Stand structure, tree growth, and silvicultural practices have a key role in wood quantity and quality. High stand density, especially in the early stages of development, promotes height growth in maritime pine stands, which shortens the period of juvenile growth of wood enabling trees to develop mature wood at early stand development stages [87, 102, 103], as well as reducing stem taper and promoting stem straightness that reduces the amount of reaction (compression) wood, thus reducing the undesirable characteristics for most wood uses [104]. However, as it is a fast-growing specie and shade-intolerant, release through non-commercial or

Figure 5.
Pinus pinaster *wood logs.*

commercial thinning should be prescribed [2]. The reasons for the early release of competition are twofold. The release will increase diameter growth and tree mechanical stability. The mechanical tree stability is frequently accessed with the h/d ratio (ratio between total tree height and diameter at breast height, with both variables in the same units). Mechanical stability is attained for h/d lower than 85 ([105] and references therein). As already referred due to its shade intolerance maritime pine individuals, when in dense stands lose their lower branches [2] whether due to shading or branch abrasion, originating the crown regression and reduction of growth [25]. Two structure indices can be used as proxies of potential photosynthetic ability, vigor, and growth: crown ratio (cr: percent of crown length in relation to total height), which is also used for mechanical stability assessment; and linear crown ratio (lcr: percent of the crown in relation to stem diameter). For good vigor and growth cr ≥ 30% and lcr > 50%, while for a good mechanical stability cr ≥ 50% ([105] and references therein).

Spatial tree arrangements have also a determinant role in wood quality. In irregular spacing, especially in dense stands, trees can develop eccentric and tortuous or leaned stems, which reduce mechanical stability, in particular to wind and snow, and depreciate wood quality due to compression wood [85, 104].

Stem taper determines the quantity and quality of wood. Theoretically, trees in free growth tend to have stems more conical while those with narrower spacing tend to be more cylindrical. Also, maximum radial growth is higher near the crown base where carbohydrates are more available due to mechanical stress [85]. Thus, density should be suited to the development of cylindrical stems. Wood quality is also determined by the presence of branches and juvenile wood. Early pruning indicated for maritime pine [2] enables to increase in the length of the cylindrical stem, reduces the knotty stem core, and promotes the formation of mature wood [2, 85, 87, 102, 103]. Pruning in the early stand development stages, with few high-intensity interventions enables an easier and faster recovery of the tree growth. The goal is to attain a knotty stem core of 1/3 or less of the diameter at breast height at the end of the production cycle [2].

Annual radial growth and its variability also determine the quantity and quality of timber. The goals are attaining a radial growth as large and as constant as possible, that maintains good wood technological properties. Thinning, redistributing the growing space by the better-suited trees that are foreseen to reach the end of the production cycle, enables to achieve the two aforementioned goals. Thinning from below and selective (Schädelin) thinning can be used [2]. In the former the trees removed are predominantly the dominated ones, thus maintaining the upper canopy. The latter is characterized by the selection of the future trees which are released from completion in thinning. This results in a trend towards higher growth rates in the latter [77]. Regarding thinning intensity, the higher the larger the radial growth, but also increases annual radial growth variability [77]. Thus, the option is between thinning of lower intensity and higher frequency or of higher intensity and lower frequency.

When the objective of forest stands is the production of quality wood, it is advisable that they be installed with reduced spacing. With this practice, the height growth is promoted (in detriment to diameter growth), in order to release the influence of the crown at the lower levels of the stem as soon as possible, reducing the amount of juvenile wood in the stem and promoting the early development of the mature wood (of better quality) in the lower levels of the stem [87, 102, 103], which are the most valuable due to their larger dimension in diameter. At the same time, the stem taper is reduced and its straightness is increased, thus also reducing the amount of compression wood, which presents undesirable characteristics for most wood uses [104].

A profile of a radial section of maritime pine wood is shown in **Figure 6**.

3.5 Growth rate *vs.* wood quality

Given the great importance of the effect of the growth rate on wood quality, this topic has been studied for a long time, without, however, maintaining a great controversy, even allowing any bibliographic review to be forwarded to support any of the preconceived views. Initially, it was generally accepted that, in softwoods, rapid growth was associated with low densities, but this idea was based on a simple analysis of the cross-section of the stem by comparing the wide rings with low density, located in the center of the tree (juvenile wood), and the narrow rings with high density, located close to the bark (mature wood). However, the effects of ring width and age were confounded, so that most of the problems thought to be related to wide rings were, after all, due to the age of wood formation, that is, due to juvenile wood versus mature wood [106]. Regardless of the ring width, the juvenile wood is characterized by presenting a low density, which contrasts with the high density of the mature wood. Although the juvenile wood of softwood normally presents wide rings, the narrow rings of the juvenile wood also have low densities, as well as the wide rings of the mature wood show high densities [102]. Thus, the true effect of growth rate on density (as well as on other properties) can only be well evaluated in rings of the same age [106]. Currently, it is consensual that it is the occurrence of juvenile wood (age of the growth rings) and not the growth rate in diameter (ring width) that produces the worst quality wood.

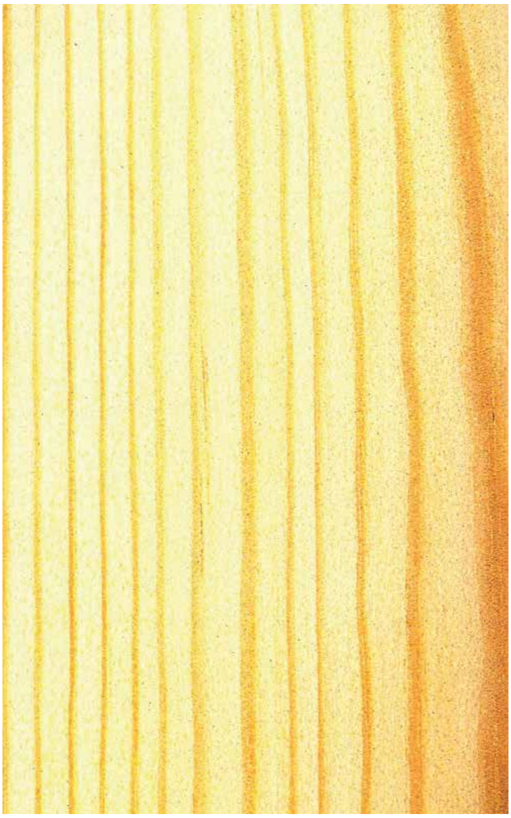

Figure 6.
Radial section of Pinus pinaster *wood.*

Numerous studies carried out with resinous species in Portugal and Spain have repeatedly demonstrated the absence of correlation between ring width and wood quality characteristics [107–117] which are sufficiently clear to stop fearing, for this species growing in these regions, any hypothetical antagonism between the vigor and the wood quality. In this regard, also worth mentioning the work carried out by Fernandez-Golfin and Diez [107] on the influence of the ring width on the wood density and other physical-mechanical properties of wood in different species (among which *P. pinaster*). In addition to corroborating the reduced predictive capacity of ring width for wood density, the authors draw attention to the fact that the first research teams on wood technology were North European, so the most widespread wood quality standards came from studies carried out in these latitudes with slow and homogeneous species, as a result of reduced interannual variability. However, according to these authors, the woody material produced in Southern Europe is characterized by an enormous variability in the ring width, essentially induced by the great variability of precipitation, which, in this region, is the main limiting factor for growth. Thus, "The wood of these species and origins must be classified according to standards that take into account their growth characteristics and not using standards made to classify other species and/or provenances. In this sense, the use of the ring width as a limiting factor of wood quality (imposed by many European classification standards) only results in an unfounded technical barrier to wood from fast-growing species and/or from European southern climates, and open the doors to slow-growing species from more northern regions" [107].

4. Growth models, simulators, and decision support systems (DSS) for maritime pine (*P. pinaster*)

The importance of the maritime pine, both in area and yield, has led to the development of a large number of growth and production models to support the management of this forest resource. The first growth models for maritime pine - in the form of Yield Tables - were developed in Portugal, for the Leiria National Forest, by Santos Hall [118], and in Spain, in the 1940s, by Echeverría and De Pedro [119] in the Atlantic area. In France, the production tables developed by Décourt and Lemoine [120] for the pinewoods of the SW region (Landes) were the first models published for the specie. Significant development of models followed, attesting to the interest shown in this field of modeling applied to the species by researchers and technical experts. The evolution of the models since the production tables reflected the state of the art in the respective research area at the time, and documents the contemporary approach to forest growth prediction. In general, the models that have been proposed are empirical, at the stand or tree level, aiming at the application to pure and regular *P. pinaster* stands. The Dryads model [121], for uneven-aged, pure or mixed stands of *P. pinaster* and hardwoods (*Castanea* spp. and *Quercus* spp.), the PBIRROL model [122], for uneven-aged stands, should be highlighted here, due to their distinctive application, as well as the tests performed with the hybrid models, physiologically based of FOREST-BGC [123] and 3-PG [124], calibrated for the species by Lopes [125] and Alexandre [126]. Additional information about growth models can be found in Fonseca [127] and Bravo et al. [128]. Fonseca [127] presents a list of 30 models developed for the species in Portugal, and Bravo et al. [128] summarize the main models developed for the Atlantic and the Mediterranean maritime pine forests in Spain. The FORMODELS database (available at http://www.iefc.net/formodels_database_forest_modeles_liste/**) contains** a comprehensive list of 20 models developed for the species for different ranges

Simulator	Reference	Main characteristics and access (code)	Platform
PBRAVO	[131, 132]	Stand level with disaggregation by diameter classes (Weibull function)	CD Rom (Pbravo vs. 2.0).
ModisPinaster	[32, 133, 134]	Stand level with disaggregation by diameter classes (Johnson SB)	CAPSIS (http://www.inra.fr/capsis)
PBIRROL	[22, 122]	Tree level, distance-dependent	StandSim.dd simulator (http://www.isa.utl.pt/cef/forchange/fctools)
PINASTER	[135] Additional references in sIMfLOR	Tree level, distance-dependent.	StandSim.dd simulator (http://www.isa.utl.pt/cef/forchange/fctools)
FlorNExT	[136]	Online application developed for the simulation of the growth and production at stand level. Combines several models developed for the species. Additional references in FlorNExT. Application of ForesMTIS. Web product	ForesMTIS. (http://flornext.esa.ipb.pt/)
GesMO	GesMo 2005 1.0 na§ GesMO 2.0 [137]	Growth simulator and product classification for several species, including maritime pine	CD-Rom.
SIMFOR	na§	Simulator for maritime pine located on Qforestry Platform for results transfer related to quantitative methods for forest management	Qforestry (https://www.qforestry.com/)
SIMANFOR	[130, 138]	Support system for the simulation of sustainable forest management alternatives which includes modules for maritime pine	SIMANFOR (www.palencia.uva.es/SIMANFOR)
PP3	[139]	Tree level, distance-independent	CAPSIS (http://www.inra.fr/capsis)

Simulator	Reference	Main characteristics and access (code)	Platform
Lemoine	[140]	A stand growth model	CAPSIS (http://www.inra.fr/capsis)
Afocelpp	na$^{\$}$	Tree level, distance-independent	CAPSIS (http://www.inra.fr/capsis)
Pinus pinaster	na$^{\$}$	Tree level, distance-independent. Adaptation of PP3, for the integration of spatialized processes	CAPSIS (http://www.inra.fr/capsis)
SilmarS	na$^{\$}$	Growth model	CAPSIS (http://www.inra.fr/capsis)

$^{\$}$*Specific reference not available; see the Web reference for details.*

Table 1.
Simulators and web products for Pinus pinaster.

of applicability in Portugal, Spain, and France, most of them referring to growth models and a few of other categories (biomass, mushrooms and fire behavior).

In this section we identify the simulators available for the species, presenting the references as to authorship or their reference documents and availability to users. Some of the models are hosted on platforms, namely, the CAPSIS (Computer-Aided Projection of Strategies in Silviculture) platform, see [129], the platform "Qforestry" (Quantitative forestry), the web-based application to simulate alternatives for sustainable forest management SIMANFOR [130], and the "sIMfLOR" platform, where the StandSim.dd simulator is located (**Table 1**) [141].

Although each model has its own specificities, the models produced to describe the dynamics of growth and several of them make it possible to anticipate the results of silvicultural options or management scenarios, according to predefined objectives or those to be achieved.

To support forest management, optimization models are used, usually anchored in Decision Support Systems (DSS), with the objective of obtaining optimal solutions for a given objective - usually wood production - subject to a set of constraints. Examples of optimization models for *P. pinaster* are found in Pasolodos-Tato [142], Fonseca [143], Rodil [144], and Petucco et al. [145]. In terms of supporting decision, Costa et al. [146] and Garcia-Gonzalo et al. [147] present case studies of DSS to generate management plans aimed at the production of wood for common lands and national forests, respectively, in Portugal. Other references are Falcão and Borges [148] and Garcia-Gonzalo et al. [148, 149].

5. Wood traits

5.1 Anatomy

Concerning the anatomical characterization, *P. pinaster* wood shows particularly longer tracheids than most resinous woods, which gives it great axial cohesion during its mechanical performance in use. For example, while *P. pinaster* wood presents an average tracheids length of 4.35 ± 0.50 mm [150], *P. nigra* and *Cupressus lusitanica*, also growing in Portugal, present average values of 3.74 ± 0.45 mm and

1.60 ± 0.16 mm, respectively [151, 152], *P. sylvestris* 1.73 ± 0.12 mm in Finland [153] and *Picea abies* with average values of ~2.75 mm [154], much lower than *P. pinaster*. Another important anatomical wood feature is the dimension of the lumen diameter of the earlywood tracheids, which in maritime pine is approximately 33 μ, a significantly higher value than that of *Picea abies* (27μ) and *P. sylvestris* wood (29 μ) [155]. This characteristic is reflected in the good performance of *P. Pinaster* wood in its drying behavior and preservation treatments.

5.2 Physical properties

The usual air-dry wood density values of approximately 0.566 g/cm^3 in 30-year-old trees are worth mentioning [156], but which can reach average values of 0.657 g/cm^3 at 70 years old [150]. These values are identical to those of *P. nigra* (0.588 ± 0.096 g/cm^3) [116, 117] and *P. sylvestris* (0.588 ± 0.101 g/cm^3) [114, 115] with identical ages and growing in Portugal, but higher than *P. sylvestris* wood from Sweden, France and the Czech Republic (0.391–552 g/cm^3) [157–160], *Picea abies* (0.410–516 g/cm^3) [157], and *Abies balsamea* (0.351 g/cm^3) [161].

Another important aspect is that the difference between the wood density of the earlywood and the latewood is not very high, which results in a considerable homogeneity of density within rings [108, 151], with very advantageous repercussions in terms of its workability, namely in its transformation into sheet to plywood and veneer and in the easiness of receiving connection elements (e.g., nails, screws).

The fact that *P. pinaster* wood has a relatively high density, has consequently a great dimensional instability caused by the gain or lose water during the wood drying (sorption/desorption processes), which results in tangential shrinkage values (T) between 9.1% at 10.1%; Radial (R) between 4.7% and 6.0%; Axial (L) between 0.0% and 1.0% and volumetric (V) between 14.5% and 16.7% [156, 162]. This aspect may be particularly critical in situations where wood is used outdoors, heavily exposed to adverse weather conditions. Comparatively, in softwoods it is common to find lower shrinkages, whose mean T values are usually between 5.6% and 8.3%; R between 3.1% and 5.3%, and V between 9.4% and 13.4% [163, 164]. In this way, it is imperative not only special care during the drying process but also that it only be applied after its moisture content is stabilized in the air. Additionally, it is also recommended to periodically apply insulating products (*e.g.*, paints, varnishes) to reduce these shrinkages [87, 103].

5.3 Chemical properties

In relation to chemical properties, the wide range of studies carried out on this theme has been unanimous in demonstrating a reduced variability, not only between different conifers species but even between trees of the same species. This lack of variability is notable not only in terms of variations in the macromolecule contents (cellulose, hemicelluloses, and lignin), but also in terms of the elemental chemistry. The only difference that is sometimes identified is related to the extractive content of some species, whose range values are usually from 1.5 to 5% [165–167]. In the case of maritime pine in Portugal, it usually presents relatively higher contents, between 4.2% and 9.6% [113, 168–170].

Even so, these values for *P. pinaster* are lower than those reported for the *P. sylvestris* (10.7–15.4%) and *P. nigra* wood (6.6–12.9%) growing in Portugal [115, 117]. In terms of the use of *P. pinaster* wood, these high extractive values give it some natural resistance to biodegradation (but do not prevent the need to apply preservative products in situations of outdoor use) but may cause some problems in surface finish operations.

Regarding the elementary chemistry contents, several studies have shown that the woody biomass of the *P. pinaster*, not only contain high heating value (HHV), between 20.15 and 21.60 Mj/kg, but also low undesirable elements contents, such as N, S, K, Na, Ca, Mn, Ni, Cr, Cu, F, Cl, and ashes [171–177]. Thus, the *P. pinaster* wood is one of the most suitable types of biomass for energy purposes, namely through combustion processes, given the high HHV and the low risk of sintering and corrosive effect of chloride salts and HCl on metal parts in furnace and boiler, that occurs when the halogen elements (F and Cl) are high [178–186]. Likewise, the low values of N and S also indicate a reduced risk of formation and release to the atmosphere of NOx and SOx [180, 187–190].

5.4 Genetics and breeding

Although the studies on genetic improvement of *P. pinaster* in Portugal had started in the 60s of the last century, they were focused on the characteristics of growth, form, and resistance to pests and diseases, and only in the last 25 years did the first study on the genetic control and improvement of the wood qualitative characteristics. At the moment, there is enough knowledge to recognize the existence of high genetic variability (essential to ensure good genetic gains through an improvement program) for some wood characteristics. For example, there was a high genetic control of the characteristics associated with wood density (heritability between 0.60 and 0.98), much higher than that verified for the growth characteristics in diameter (between 0.15 and 0.17), height (0.34), as well as for other wood features, such as lignin content (0.34), Radial Modulus of Rupture (0.34) and Radial Modulus of Elasticity (0.30) [108, 110, 111, 113]. Furthermore, when analyzed separately, the earlywood (formed in spring) exhibits much greater genetic dependence and is controlled over several years by the same set of genes, being the one that better results will provide in the future selection and improvement programs. In the opposite situation, the latewood, showing the lowest and most unstable heritability values, reveals that this type of wood is more strongly affected by environmental conditions than the earlywood [108, 110, 111, 113].

With regard to ring width, no adverse genetic correlations were detected between this and the wood density components. The fact that ring width is genetically and consistently positively correlated with the ring density, earlywood density, latewood percentage, and negatively with the heterogeneity index, allows us to contest, once again, the erroneous idea, but unfortunately still deeply rooted in the thinking of many researchers and wood users, that trees with higher radial growth (higher ring width) produce lower wood quality, namely lower density and latewood percentage in xylem [109, 110, 113].

These results should be sufficiently enlightening for us not to fear, for this species, any possible antagonism between the vigor and wood quality. On the contrary, it is expected that selection by the ring width will have a correlated effect in a slight increase in ring density, earlywood density, and latewood percentage (which should make it possible to reconcile good radial growths with high density), but not being accompanied by any significant changes in the latewood density, which will indirectly allow to increase the homogeneity of the growth rings. This fact is one of the most valued attributes by some of the wood processing industries. For example, the greater the homogeneity within the rings, the easier and more profitable will be the production of veneers, the greater its mechanical strength, the easier it receives the connecting elements (nails and screws), and the lower the risk of wood cracking [109, 113].

One of the places where the genetic improvement of P. *pinaster* is most advanced is in Australia, which began in 1957 and is currently in its fourth phase. The first phase was the establishment of a preliminary test of provenances that took place between 1964 and 1984 which revealed that in the growing conditions of West Australia, the provenances from Leiria (Portugal) were the most vigorous, confirming, once again, the superiority of the Atlantic provenances for growth [191]. The development in height and diameter at 10 and 20 years old was much higher in the 2 origins from Leiria, compared to those from Corsica, Landes, and Italy and, in terms of volume, the origins from Leiria presented a value greater than twice that of any of the other provenances. Furthermore, the provenances from Leiria were also the most resistant to drought (0.8% mortality, compared to 9.7% for the Landes and 10.1% for Corsica), but little to frost and with frequent stem bifurcations. The provenances from Corsica were superior in the stem straightness, while those from Leiria did not differ significantly from those from Spain and did not show a good performance in this parameter.

In the second phase of the improvement program, an attempt was made to combine, in the same individuals, the vigor characteristic of the provenances from Leiria with the stem straightness of those from Corsica, having crossed these two provenances. However, the hybrids obtained by this cross kept these 2 characteristics apart in the same individuals: either a high vigor, or a good stem configuration, but never both, simultaneously [191].

Faced with this setback, the next phase aimed to improve the stem shape while maintaining its high vigor, using material from 86 selected trees in the Leiria pine forest, which provided considerable genetic gains. According to Butcher and Hopkins [192] and Hopkins and Butcher [193] at this stage of the program, an increase in total volume production of +36% was obtained, which represents, by itself, an average increase of about 3.5 m^3 ha^{-1} $year^{-1}$ and which, complemented by a significant improvement in the stem quality by increasing their straightness by around 40% and by reducing the size of the branches by 25%, allows for an even greater increase in the total volume of usable wood.

For the fourth phase of the program, which is still in progress, the main objectives were to improve the characteristics of the branches (reduction of the insertion angle and size) and to increase the wood density, having been selected the best individuals from the best families obtained in the previous phase of the program that showed good configuration of the stem and crown, and whose average density of juvenile wood was equal to or greater than 0.430 g/cm^3 [193–195].

Thus, the current knowledge about the properties and characteristics of *P. pinaster* wood allowed to identify it as a type of wood with potential for a wide range of uses, which go beyond those with less added value (packaging, pallets, and briquettes). In fact, this wood has suitable characteristics for more noble applications, such as structural applications, floors, carpentry and furniture, veneer, particleboard and plywood, poles, and sleepers.

6. Conclusions

Maritime pine is a plastic species widely distributed. Its traits and stand structures as well as the quantity and quality of its wood allow a wide range of uses. The stands are managed for wood, non-woody products, and services, thus recognizing its importance both economical and as a provider service demanded by society, thus contributing to its well-being.

The large representation of the species, particularly in southern Europe, has allowed advanced research on silvicultural systems and cultural practices, and their effects on wood properties, providing clarification on less well-perceived aspects of wood quality, particularly when considering the development of the species in the

Mediterranean region. In parallel with silvicultural studies, several growth models and simulators have been developed and proposed to support management.

The challenges facing the species in the future are known, including severe weather conditions, especially drought, rural fires, storms, pests, and diseases. In addition, the systems are under pressure due to the high demand for woody material. From the extensive review carried out on maritime pine, it is noticed these challenges are part of research conducted or underway and of joint initiatives through international research projects (e.g., ForManRisk, https://formanrisk.eu/) to ensure the definition and update of management guidelines for the sustainability of maritime pine systems in the long term.

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Conflict of interest

The authors declare no conflict of interest.

Author details

Teresa Fidalgo Fonseca[1,2,3]*, Ana Cristina Gonçalves[4] and José Lousada[1,5]

1 Department of Forestry Sciences and Landscape Architecture (CIFAP), University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

2 Forest Research Center (CEF), School of Agriculture, University of Lisbon, Lisboa, Portugal

3 IUFRO Division 1 - Unit Ecology and Silviculture of Pine, Austria

4 Department of Rural Engineering, School of Sciences and Technology, MED—Mediterranean Institute for Agriculture, Environment and Development, Institute of Research and Advanced Education (IIFA), University of Évora, Évora, Portugal

5 Center for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

*Address all correspondence to: tfonseca@utad.pt

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Chapter 2

Complexity of Regeneration Dynamic at the Ecocline between Mixedwood and Coniferous Domains of the Southernmost Boreal Zone in Eastern North America

Yassine Messaoud

Abstract

To explain the ecocline between the southern mixedwood and the northern coniferous bioclimatic domains dominated, respectively, by balsam fir (*Abies balsamea* (L.) Mill.) and black spruce (*Picea mariana* (Mill.) B.S.P.), 59 field sites and 7010 sample plots (from the Quebec Ministry of Forests, Wildlife, and Parks), with no major disturbances, were selected throughout the two bioclimatic domains. Regeneration (seedlings and saplings), mortality (difference between seedlings and saplings) of balsam fir, and black spruce (saplings) were examined, accounting for parental trees, main soil type (clay and till), summer growing degree-days above 5° C (GDD_5), and total summer precipitation (May–August; PP_MA). Balsam fir regeneration was more depended on parental trees and soil type than black spruce. Balsam fir mortality was related to seedling competition, species composition of the canopy, and the soil type. GDD_5 and marginally PP_MA were beneficial and detrimental for respectively balsam fir and black spruce regeneration. The ecocline mixedwood/coniferous bioclimatic domains was attributed to a northward gradual decrease of balsam fir regeneration and increase of its mortality, due to cooler temperatures, shorter growing seasons, and decrease of the parental trees. However, balsam fir persists above this ecocline, where parental trees populations and good establishment substrates occur.

Keywords: balsam fir, black spruce, natural regeneration, mortality, ecological conditions, climate change

1. Introduction

In North America, the southern limit of the continuous boreal zone decreases in latitude from Alaska eastward [1, 2] and reaches its southernmost (*circa* 48°N) in eastern Canada, between eastern Ontario and western Quebec. At this point, this is where the boreal zone reaches its most southerly limit worldwide, except for pockets at high elevations [3]. In Quebec, the area is composed of two bioclimatic domains that are characterized by different late-successional species in mesic sites: the southern balsam fir—paper birch (*Betula papyrifera* Marsh.) bioclimatic domain (hereafter, referred to as mixedwood forest) with some species reaching their northern distribution limit, such as sugar maple (*Acer saccharum* Marsh.), yellow birch (*Betula alleghaniensis* Britton), red pine (*Pinus resinosa* Ait.), white pine (*Pinus strobus* L.) and red maple (*Acer rubrum* L.) [4, 5], and the northern black spruce—feather moss bioclimatic domain (hereafter, referred to as coniferous forest [6]. Trembling aspen (*Populus tremuloides* Michx.), paper birch, and jack pine (*Pinus banksiana* Lamb.) are abundant immediately after fire in both bioclimatic domains.

The ecocline between the mixedwood and coniferous bioclimatic domains occurs at *circa* 49°N, which represents the shift in dominance for the two species instead of their range limit. Indeed, black spruce extends farther south into the temperate forest zone, where it reaches its southern limit at *circa* 40°N [7], while balsam fir reaches its northern limit at 54°N [8].

Balsam fir and black spruce have contrasting ecological traits. In fact, balsam fir is more shade-tolerant but less cold-tolerant than black spruce. Furthermore, balsam fir does not have seed bank, while black spruce cones containing seed remain in the canopy for several years [9]. In addition, balsam fir is less resistant to fire than black spruce given that the former has a thinner bark [10]. Therefore, extreme fire is not conducive to the regeneration of balsam fir, and the availability of seeds depends to a large extend on the living parental trees in protected areas from fire. Moreover, balsam fir is less adapted to saturated soil water conditions compared with black spruce [11].

Regeneration dynamics are closely dependent on seed availability and recruitment potential, which may be limited by many factors [12, 13]. Tree seedlings, the first stage of regeneration, are specially dependent on seed source (parental trees) and soil substrates that are suitable for their establishment [14]. Since their root systems are shallower and less extensively developed, seedlings may be unable to explore and exploit soil resources compared with later regeneration stages, which make them particularly sensitive or susceptible to spatiotemporal variability in microenvironments and the variability of regeneration niches [15].

Climate is well known to directly and indirectly limit regeneration dynamics [16, 17]. As a direct effect, low temperatures and diminished precipitations can reduce seedling survival in northern Holarctic forests [18, 19]. As indirect effects, low temperature reduces the organic matter decomposition (low soil fertility) and soil evaporation (water surplus and paludification), unsuitable substrates for establishment of many species [20]. In addition, forest fires also influence regeneration, depending upon the species' fire tolerance [21].

At the leading woody species range expansion, regeneration becomes more temperature-limited as latitude and elevation increase, thereby decreasing seedling abundance due to lower seed inputs and higher mortality that prevents them from establishing further northward [22]. Yet, fewer studies have been conducted at the transition between abutting, closed-canopy forest ecosystems [23]. In North America, some studies on tree regeneration dynamics [23, 24] have been conducted at the ecocline between the boreal and temperate zones that lies further to the south. The former is dominated by balsam fir, while the latter is dominated by sugar maple. These studies showed that although balsam fir did not reach its tailing range [2], regeneration is better adapted to colder climate, yet it is limited by sugar maple litter thickness. In contrast, sugar maple is limited at its leading range by soil acidity and cold temperature.

The objective of the study is to determine if the location of the ecocline between the mixedwood and coniferous bioclimatic domains is explained by the contrast regeneration dynamic between the two dominant tree species. We expect a decrease of balsam fir regeneration and increase of its mortality in the coniferous bioclimatic domain compared with mixedwood domain, due to unfavorable climate and substrate conditions. What makes this study unique is that it is conducted not only between two forested areas, but also at the shift between two species dominance. In addition, we verify to what extent the maintenance of balsam fir and black populations is linked with parental trees and regeneration potential driven by climate and site conditions. This chapter was derived from the two projects conducted by Messaoud *et al.* [25, 26].

2. Study design

2.1 Survey area

The area was located in western Quebec (Canada) and is a part of the Quebec and Ontario Clay Belt, formed by lacustrine sediments left by the former proglacial lake Barlow-Ojibway ([27]; **Figure 1**). The altitude ranges from 300 and 400 m asl, with low hills scattered in a flat landscape. The area has a continental climate, with cold winters and warm summers. Yet, climate differs between the two bioclimatic domains (**Table 1**).

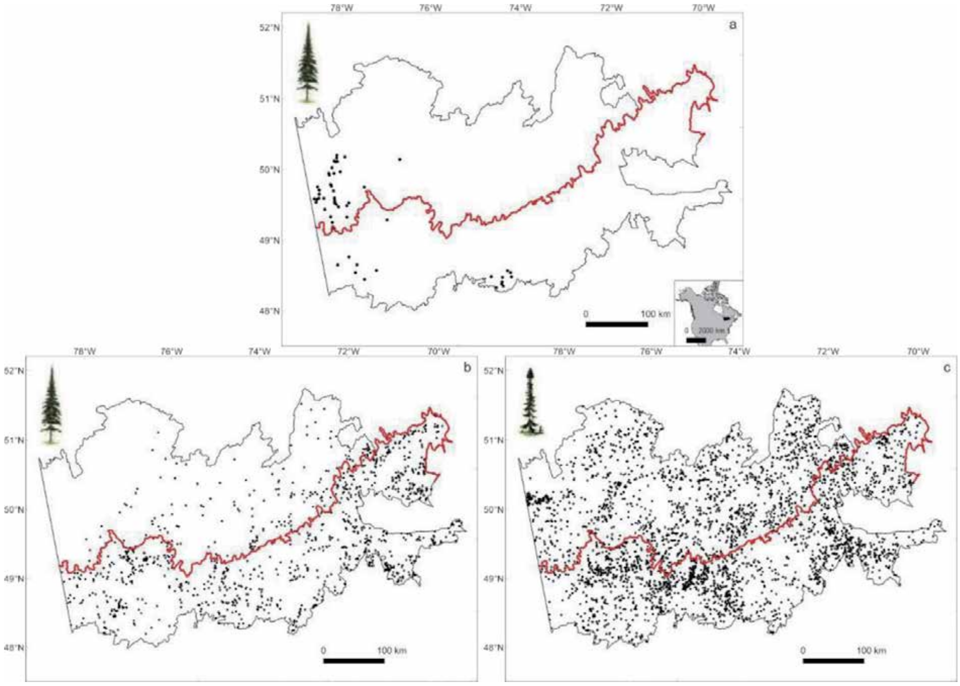

Figure 1.
Study area showing the field sites (a) and sample plots for balsam fir (b) and black spruce (c) stands that were established for the collection of forest inventory data by Ministry of forest, fauna, and parks of Quebec (MFFPQ). The bold red line that runs from east to west depicts the boundary between the southern mixedwood and northern coniferous bioclimatic domains in western Quebec (after [6, 25, 26]).

	Bioclimatic domains	
	Mixedwood	**Coniferous**
Mean annual temperature (°C)	0–1	-2.5 to 0
Length of the growing season (days ≥ 5°C)	150–160	120–155
Total annual precipitation (mm)	800–1200	700–1000
Percentage of precipitation falling as snow	40–45	25–50

Table 1.
Climatic variables for the western bioclimatic domains.

2.2 Sampling design

2.2.1 Field design

Fifty-nine closed-stand sites were selected along a latitudinal gradient throughout the ecocline between the mixedwood and coniferous domains (**Figure 1a**). These sites are located on public land, easily accessible from nearby roads, with no major disturbance, had a moderate moisture regime [28], and the surface deposits consist of either clay or till.

In each site, a transect was established perpendicular to the nearby road, where five circular plots (40 m^2) were randomly selected independently from each other. Within each plot, balsam fir dynamics were surveyed by counting the number of seedlings (diameter at breast height, DBH < 2 cm), saplings (DBH between 2 and 9 cm inclusively), and mature trees (≥10 cm DBH). DBH of the mature trees was measured. The data from all five plots in each site were combined to compute an average number of seedlings, saplings, and mature trees to account for plot variability. These data were subjected to a basic negative exponential model, which may properly reflect the depletion of balsam fir populations over a very short period of time, for example, from seedlings to saplings [29]. This model assumes a consistent balsam fir mortality rate for different age classes at a given site. As a result, we computed the difference between seedling and sapling numbers, which were also reported as percentages, to assess absolute mortality between two age classes in each site.

2.2.2 Inventory design

A total of 7010 sample plots (400 m^2 each) characterized by closed-stand and no major disturbance were provided by the Quebec Ministry of Forest, Wildlife, and Parks (MFFPQ) and used to test the natural regeneration of balsam fir and black spruce in the mixedwood and coniferous domains (**Figure 1b** and **c**; [30]). Balsam fir was more common in the mixedwood bioclimatic domain than black spruce, which was more common in the coniferous ones. Within each sample plot, balsam fir and black spruce saplings (2 cm ≤ DBH < 10 cm) were counted and measured including all mature trees, irrespectively for species (DBH ≥ 10 cm) and downloaded from the database, as well as with latitude, longitude, elevation, and soil type (clay or till, which are the dominant parent materials in both forests). The basal area (m^2/ha) of mature trees in each plot was estimated and then converted to a percentage of total mature trees. To classify the evolution of the sites toward black spruce or balsam fir, a threshold was used as follows: when the proportion is 60% or more of black spruce, the site is considered to be developing toward black spruce dominance. Whereas when the proportion of balsam fir was ≥40% of the coniferous component, the site is considered to be developing toward balsam fir dominance. This threshold was set based on the competitive ability and shade tolerance of the two species; under similar condition, balsam fir is generally more competitive and more shade-tolerant than black spruce [2, 10]. In addition, disturbed sites and sites with <20% balsam fir or spruce in the canopy and jack pine sites were excluded from the analyses [11].

2.3 Climate

Geographic locations (latitude, longitude, and elevation) of each sample plot or site were used to extract climate variables using the BIOSIM11 modeling software (https://cfs.nrcan.gc.ca/projects/133). Climate variables concerned cumulative growing degree-days >5°C (GDD_5) and total summer precipitation (May to August, PP_MA, mm). The GDD_5 threshold is the temperature at which plant growth begins. The influence of climate on regeneration was determined using averages of climatic parameters corresponding to climate normals for the years 1981–2010 [31].

2.4 Statistical analyses

A MIXED procedure of SAS software (V 9.1, SAS Institute Inc., Cary, N.C., USA) was used to estimate the parameters of Eqs. (1) and (2), adjusted to field and inventory data, respectively:

$$\begin{aligned} Y_{ij} = {} & \beta_0 + \beta_1 BD_{ij} + \beta_2 P_{ij} + \beta_3 S_{ij} + \beta_4 \mathrm{G}_{\mathrm{P}-ij} + \beta_5 \mathrm{G}_{\mathrm{T}-ij} + \beta_6 \mathrm{G}_{\mathrm{S}-ij} + \beta_7 BD_{ij} S_{ij} \\ & + \beta_8 BD_{ij} G_{P-ij} + \beta_9 BD_{ij} S_{ij} \mathrm{G}_{\mathrm{P}-ij} + \varepsilon_{ij} \end{aligned} \tag{1}$$

where Y is the dependent variable indicating the germination or mortality rate (in percent) for bioclimatic domain i and site j. BD indicates the bioclimatic domain, P is the sample plot, S is the soil type, G_P is the parental tree basal area ($\mathrm{m}^2\ \mathrm{ha}^{-1}$), G_S is the other tree species basal area ($\mathrm{m}^2\ \mathrm{ha}^{-1}$), and ε_{ij} ($\varepsilon_{ij} \sim \mathrm{N}(0, \sigma^2)$) is a normally distributed error term. The influence of soil on regeneration or mortality between bioclimatic domains was represented by the interaction between bioclimatic domain and soil type. The influence of parental tree basal area on regeneration or mortality was represented by the interaction between BD and G_P. For deduction purposes, the bioclimatic domain was considered as a random variable. To consider for distinction in total basal area that could differentially influence the regeneration or mortality, G_T was included in the model as a covariate. Climate influences on regeneration were evaluated employing a different model including GDD_5 and PP_MA, together with their interaction with the bioclimatic domain. All comparisons were conducted using t tests with significant difference being declared at $p < 0.05$.

$$\begin{aligned} Y_{ijk} = {} & \beta_0 + \beta_1 BD_i + \beta_2 P_{ij} + \beta_3 SP_i + \beta_4 S_{ijk} + \beta_5 \mathrm{S\_BA}_{ijk} + \beta_6 \mathrm{T\_BA}_{ijk} + \beta_7 BD_{ijk} \mathrm{S\_BA}_{ijk} \\ & + \beta_8 F_i S_{ijk} \mathrm{T\_BA}_{ijk} + \varepsilon_{ijk} \end{aligned} \tag{2}$$

where Y is the response variable indicating the sapling number for the ith bioclimatic domain, jth sample plot and the kth species. The explained fixed effect variables are BD for the bioclimatic domain, P for sample plot, SP for species, S for soil type, and S_BA for parental tree basal area. To consider for total basal area of a given sample plot, affecting somehow regeneration, total sample plot basal area (T_BA) was included as a covariate. To evaluate how regeneration was affected by a combination of numerous site factors, we also included in the model interactions between bioclimatic domain, soil type, and parental trees or total basal area. The model intercept is β_0, while β_1–β_7 are the parameters to be estimated for the explained fixed effects and their interactions. The error term, ε_{ijk}, was expected to be normally distributed ($\varepsilon_{ijk} \sim N(0, \sigma^2)$). Before analyses, and to meet the assumption of homoskedasticity of the residuals, sapling number and basal area were subjected to natural logarithmic transformation. The main categorical influences as bioclimatic domain, species, and soil type were evaluated using the PDIFF option of the LSMEANS statement. The interaction factors including categorical and continuous variables were evaluated by contrast analyses using the ESTIMATE statement. Altogether, an influence was considered significant for $p < 0.05$ based on t-tests of the fixed effects. To test the influence of climate on regeneration, relationships between regeneration and GDD_5 and PP_MA were achieved using correlation analyses.

For a given species, an index that was named "anomaly of sapling abundance" (A_{SA}) was assessed at the sample plot scale, as the difference between plot sapling abundance (SA_P) and mean sapling abundance of the total study area (SA_M). Subsequently, A_{SA} was plotted against the percentage basal area of parental trees (S_{BA}) to calculate a threshold of percent parental tree basal area from which a species maintains itself within the overall mean of the total study area (same value as the mean) or "overflows" (above this mean). A comparative method was carried out at the level of bioclimatic domain and another one controlling for both bioclimatic domains and soil type, using Eq. (3):

$$\begin{cases} A_{SA} = a + bS_{BA} \\ SA_P = SA_M S_{BA} = \text{mean threshold} \\ SA_P SA_M S_{BA} = \text{overflow} \end{cases} \tag{3}$$

In Eq. (3), the coefficients a and b are derived from a regression analysis. Prior to analyses, we tested the possible multicollinearity between the explaining variables. The results showed that for both data sources, the Pearson correlation values between different explaining variables were mostly below 0.600 or not significant ($\alpha < 0.05$; **Table A1**).

3. Results and discussion

3.1 Seedling dynamic

Balsam fir seedlings abundance was positively and strongly linked with parental tree basal area regardless for the soil type (**Figure 2a**), indicating the importance of the seed source of its parental trees, which agrees with a previous study [24]. As well, the proximity of parental trees appears to be crucial for effective regeneration, suggesting that the large size of balsam fir seed decreases their dispersal capacity [10, 32]. Furthermore, for a similar parental tree basal area, balsam fir regeneration was higher in the mixedwood than in the coniferous domain. Controlling for the soil type, balsam fir showed overall significantly higher regeneration on till than on clay soils ($p = 0.022$; **Figure 2a**). In the mixedwood bioclimatic domain, there was no evidence of the soil type effect on the regeneration. In contrast, a significantly higher regeneration was observed on till compared with clay soils in coniferous bioclimatic domain ($p < 0.001$). On till soils, basal area of other tree species had no effect on balsam fir regeneration, while it decreased and increased balsam fir regeneration in the mixedwood and coniferous bioclimatic domains, respectively ($p = 0.016$; **Figure 2b**), indicating a less suitability for balsam fir regeneration. Clay soils exhibit low temperatures and high water-holding capacity, slowing the organic matter decomposition, which leads to lower nutrient availability [33].

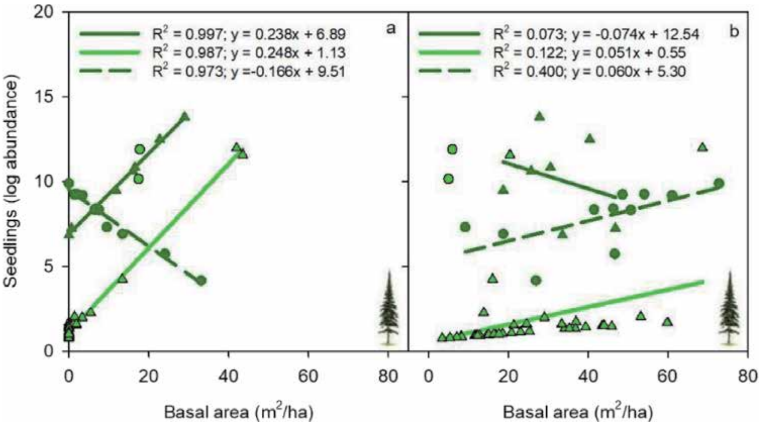

Figure 2.
Abundance of balsam fir seedlings (normal log of the number of seedlings per hectare (log abundance)) with basal area (m^2 ha^{-1}) of (a) parental trees and (b) other tree species according to bioclimatic domain and soil type. The dark green triangles and solid lines represent clay soils, while the dark green circles and dashed lines indicate till soils in the mixedwood bioclimatic domain. The light green triangles and solid lines indicate clay soils, while the light green circles indicate till soils in the coniferous domain [25].

The anomaly of balsam fir seedling abundance, representing the deviation from the mean of the total seedling abundance of the study area, was significant and positive in both bioclimatic domains except on clay soil in coniferous bioclimatic domain, where it was negative (**Figure 3**). Messaoud *et al.* [9] found in the same region a higher balsam fir reproductive capacity in the mixedwood than in the

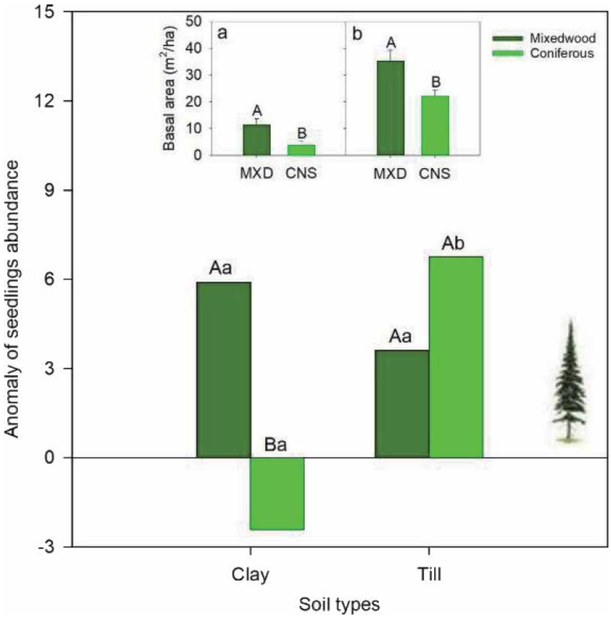

Figure 3.
Anomaly of balsam fir seedling abundance (normal log of the number of seedlings per hectare) according to bioclimatic domain and soil type. An anomaly represents a deviation from the mean of the total seedling abundance of the study area (positive = overflow, and negative = lower seedlings than the average). Inset panels indicate the basal area (m^2 ha^{-1}) between the two bioclimatic domains (MXD, mixedwood; CNS, coniferous) for (a) parental trees of balsam fir and (b) other tree species. The letters at the top of the histograms indicate nonsignificant (same letters) or significant (different letters) differences between the mixedwood and coniferous bioclimatic domains. The lowercase letters show the comparison between till and clay soils within each bioclimatic domain (modified from [25]).

coniferous domains. For a similar balsam fir basal area, there are more seed trees involved in the regeneration in the mixedwood than in the coniferous domains. However, we found a highest positive value on till soil in coniferous bioclimatic domain and secondly on clay soil in mixedwood bioclimatic domain. Controlling for the bioclimatic domain, anomaly values were higher on clay than on till soil in mixedwood bioclimatic domain, although it was not significant. Yet, the difference was strongly significant in coniferous bioclimatic domain, which corresponded with the lower parental trees basal area (results not shown). However, in mixedwood bioclimatic domain, the importance of the parental trees was higher on till than on clay soil. Thus, our findings confirm the importance of the substrate for the regeneration success, besides the strong importance of parental tree seed source (**Figure 3a** and **b**). Indeed, in coniferous bioclimatic domain, where climate is colder, clay appears to be detrimental for seedlings establishment because of its water surplus and cold temperature [33], whereas till soil became more suitable for seedling success. Note that water surplus (clay) appears to have greater effect than a possible water deficit due to more water evaporated till on the shallow seedling roots. This is obvious since forest canopy shading protects seedlings from a high soil evaporation and prevents from a high water demand of seedlings, whereas it might add the negative effect of low temperature and water surplus.

In addition, there was not only a positive relationship between balsam fir seedling mortality rate and parental tree basal area (p = 0.045), but also an unexpected higher mortality in the mixedwood compared with the coniferous domain (p < 0.004; **Figure 4a**) regardless for the soil type. Balsam fir seedling mortality declined with increasing basal area of other tree species (p = 0.020; **Figure 4b**). Thus, balsam fir mortality appears to be related to its seedling density

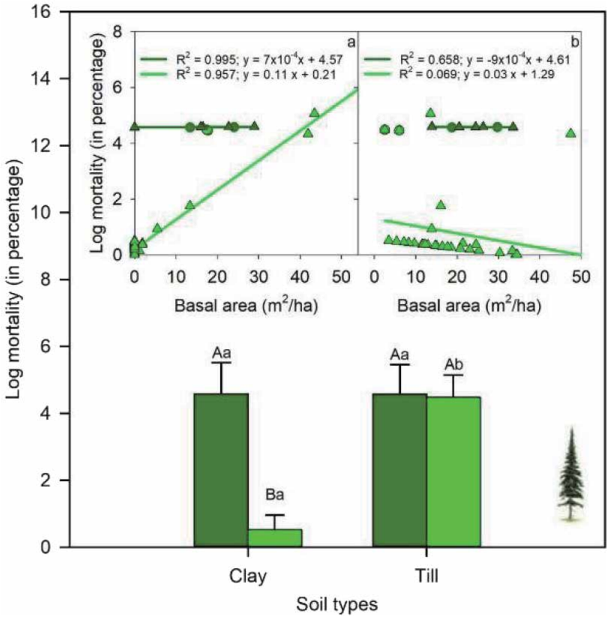

Figure 4.

Mortality rate of balsam fir seedlings according to bioclimatic domain and soil type. The inset panels indicate the basal area (m^2 ha^{-1}) *between the mixedwood and coniferous bioclimatic domains for (a) parental trees of balsam fir and (b) other tree species. The dark green triangles and solid lines indicate the mixedwood bioclimatic domain, while the light green circles and solid lines indicate the coniferous domain. The letters at the top of the histograms indicate nonsignificant (same letters) or significant (different letters) differences between the mixedwood and coniferous bioclimatic domains. The lowercase letters show the comparison between till and clay soils within each bioclimatic domain (modified from [25]).*

(competition) associated with the high seed source (parental trees). Understory shrubs and herbaceous plants are quite nonexistent in our site (personal observation), excluding any competition other than between balsam fir seedlings. In the mixedwood bioclimatic domain, seedlings are in similar shading environments; therefore, the competition between balsam fir seedlings may be for water and nutrients owing to similar quantity of soil resources being shared between a higher number of balsam fir seedlings. However, the other species had no effect or decreased mortality respectively in mixedwood and coniferous bioclimatic domains, probably due to the abundance of deciduous tree species, which may promote balsam fir establishment, notably in coniferous bioclimatic domain [34]. Another unexpected finding is that mortality rate was higher in mixedwood than in coniferous bioclimatic domains, less obvious on till soil, adding the effect of the competition between the seedlings (**Figure 4**). Moreover, higher mortality on till soils may also result from dryer conditions characterizing till soils, which easily release water during drainage or through evaporation as mentioned above [35].

The effect of climate on balsam fir seedling dynamic demonstrated a positive relationship with the growing degree-days above 5°C (GDD_5; $p < 0.001$; **Table 2**) regardless for the soil type, while total summer precipitation (PP_MA) had a negative effect on clay soil. As well, a decline of GDD_5 was observed in the coniferous bioclimatic domain compared with the mixedwood domain (**Figure A1**). Thus, lower balsam fir regeneration found in the coniferous bioclimatic domain could be linked to decrease of GDD_5 and increase of PP_MA, which worsen the water surplus of clay soil, hence showing an additional soil effect on regeneration. To test the effect of drought on regeneration, we used the interaction between GDD_5 and PP_MA [36]. The results demonstrated a positive and significant relationship with balsam fir regeneration irrespectively of the soil type, indicating a positive effect of drought. In the study area, balsam fir seedlings were in the shade of the canopy trees, where soil moisture is pretty much higher than in opened area. Furthermore, the positive effect of drought appears to prevent seedlings from a water surplus occurring on clay soil. In addition, balsam fir seedlings occurred mostly on a particular substrate such as woody mounds and thin moss cover [37], known to have a high water capacity, which may explain the absence of the PP_MA effect and the positive effect of drought on seedling abundance [38, 39].

3.2 Sapling dynamic

Balsam fir sapling abundance was significantly higher in the mixedwood than in the coniferous bioclimatic domain, while it was opposite for black spruce (**Table 3**). Moreover, this tendency was also similar for both species irrespectively of soil type. The abundance of balsam fir sapling was greater on till than on clay soils in mixedwood, whereas it was alike within the coniferous bioclimatic domain. In

Soil type	GDD_5	PP_MA	GDD_5*PP_MA
FS	**0.827****	**−0.437***	**0.857****
MM	**0.810****	−0.192	**0.897****
Total	**0.857****	−0.041	**0.912****

Correlations that significantly different from zero (p < 0.05) *are shown in boldface type:* *p < 0.01; **p < 0.001.

Table 2.
Person correlation (r) between balsam fir seedlings abundance and climate variables summer growing degree-days above 5°C (GDD_5) total summer precipitation (May to August; PP_MA), and the interaction between the two climate variables accounting for soil type (till or clay).

Species	Soil type	Bioclimatic domain	
		Mixedwood	**Coniferous**
Balsam fir	Clay	1264.86^{Aa} (91.06)	625.52^{Ba} (80.44)
	Till	1682.75^{Ab} (54.75)	620.36^{Ba} (45.87)
	Total	**1598.73^{A} (47.48)**	**621.68^{B} (39.86)**
Black spruce	Clay	2511.30^{Aa} (119.82)	3925.72^{Ba} (189.76)
	Till	2079.37^{Ab} (49.50)	2864.29^{Bb} (75.49)
	Total	**2166.21^{A} (46.38)**	**3137.25^{B} (74.83)**

Superscripts indicate nonsignificant (same letter) or significant (different letters) differences between mixedwood and coniferous domains. Uppercase letters are comparisons between bioclimatic domains, while lowercase letters are comparisons between soil types within bioclimatic domain.

Table 3.
Sapling abundance of balsam fir and black spruce (means, standard errors in parentheses) according to bioclimatic domain and soil type.

contrast, black spruce sapling abundance was higher on clay than on till in both bioclimatic domains. Our results demonstrated a significant positive role of the parental trees as seed sources on regeneration abundance for both species ($p < 0.001$). Balsam fir sapling abundance and parental tree basal area exhibited a positive relationship on both soil type, regardless of bioclimatic domain (**Figure 5a**). We noticed that although the model demonstrated no significant

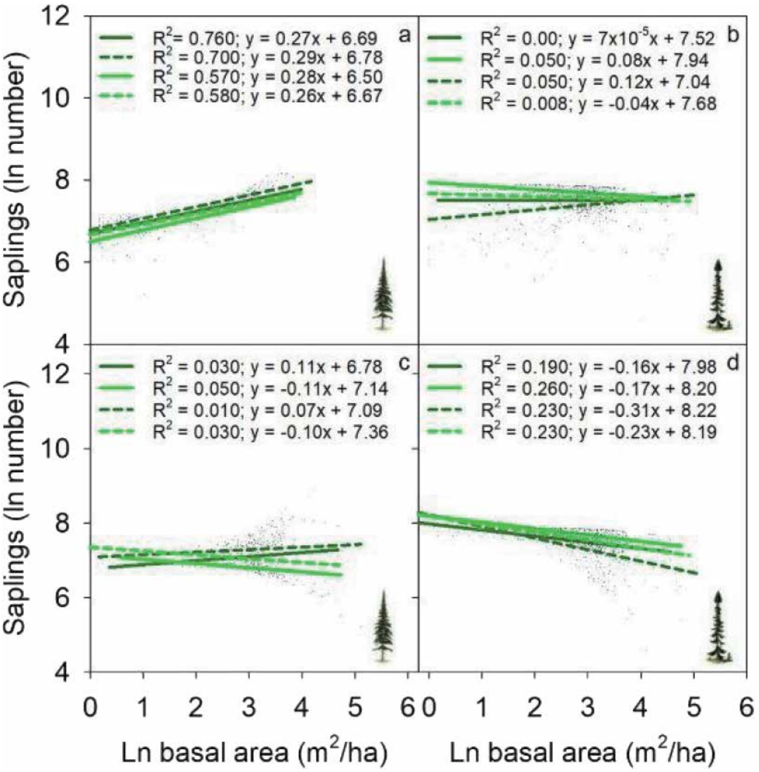

Figure 5.
Abundance of balsam fir and black spruce saplings (ln(stems/ha)) with basal for parental trees (a and b) and for the stand (c and d), according to bioclimatic domain and soil type. The dark green solid and dashed lines respectively indicate clay and till soils in the mixedwood domain, while the light green solid and dashed lines respectively indicate clay and till soils in the coniferous domain [26].

relationship, sapling abundance was greater on till than on clay soils in the mixedwood bioclimatic domain. As well, the relationship between saplings abundance and parental tree basal area was stronger in the mixedwood than in the coniferous bioclimatic domain on clay soils. With respect to black spruce, the results indicated a decline sapling abundance linked to increasing parental tree basal area, excluding on till and barely on clay soils in the mixedwood bioclimatic domain, where the relationship between sapling abundance and parental tree basal area was positive (**Figure 5b**). The relationship between sapling abundance and parental tree basal area was also stronger on clay soils. Furthermore, greater abundance of black spruce sapling arose in the coniferous domain (p = 0.027). For balsam fir and regardless of soil type, total basal area positively impacted sapling abundance in the mixedwood, but negatively influenced it in the coniferous bioclimatic domain, although the general distinction prevailed nonsignificantly (**Figure 5c**). The relationship between black spruce sapling abundance and total basal area was negative, irrespectively of soil type or bioclimatic domain (**Figure 5d**). The negative impact of total basal area on regeneration seemed to be significantly stronger on till soils in the mixedwood ($p < 0.001$) and coniferous (p = 0.003) bioclimatic domains. In differentiating both bioclimatic domains, the negative influence of total basal area on abundance of black spruce sapling was more grounded in the coniferous than in the mixedwood bioclimatic domain, regardless of the soil types ($p < 0.001$). This indicates that with the increase of the parent tree base area, the influence of soil type on black spruce regeneration is greatly reduced, which may be due to the convergence of soil temperature and organic layer thickness conditions between the forest domain and the soil type. Therefore, stands dominated by black spruce seem to change its own microclimate and soil conditions by increasing soil moisture and lowering the soil temperature, which is conducive to promoting its seedling abundance. However, on clay soils in the coniferous bioclimatic domain, black spruce regeneration is more likely to be negatively affected, due to soil moisture saturation and soil temperature decrease, which both lead to paludification [31, 40].

Figure 6 shows a different regeneration pattern between balsam fir and black spruce. Balsam fir parental tree basal area was higher in mixedwood bioclimatic domain, while the opposite was true for black spruce in coniferous bioclimatic domain, which overlapped with regeneration abundance. Again, the presence of nearby seed trees has been previously reported as an important factor explaining regeneration abundance [41, 42]. For comparable parental tree basal area between both bioclimatic domains, regeneration for balsam fir was higher in the mixedwood domain, with black spruce demonstrating higher regeneration in the coniferous domain. As well as to parental tree influences on regeneration abundance, their potential seed production appears to play a crucial role in regeneration. As noticed before, balsam fir of comparable basal area has been demonstrated to produce fewer seeds in the coniferous than in the mixedwood bioclimatic domain [9], resulting in lower subsequent regeneration in the coniferous domain. In the same study, Messaoud *et al.* [9] found that black spruce showed similar seed production between the two bioclimatic domains. Thus, the regeneration pattern seems to be related to the reproductive capacity of both species. However, the reproductive capacity is not enough for the regeneration success. Indeed, balsam fir saplings abundance is still below the average of the total saplings in the study area on the clay soil, more pronounced in coniferous than in mixedwood bioclimatic domains, while it was only positive in mixedwood bioclimatic domain on the till soil. For black spruce, regeneration abundance was positive regardless of the bioclimatic domains and soil type, except on till soil in mixedwood bioclimatic domain. The negative effect of clay soils on balsam fir has already been reported in a previous

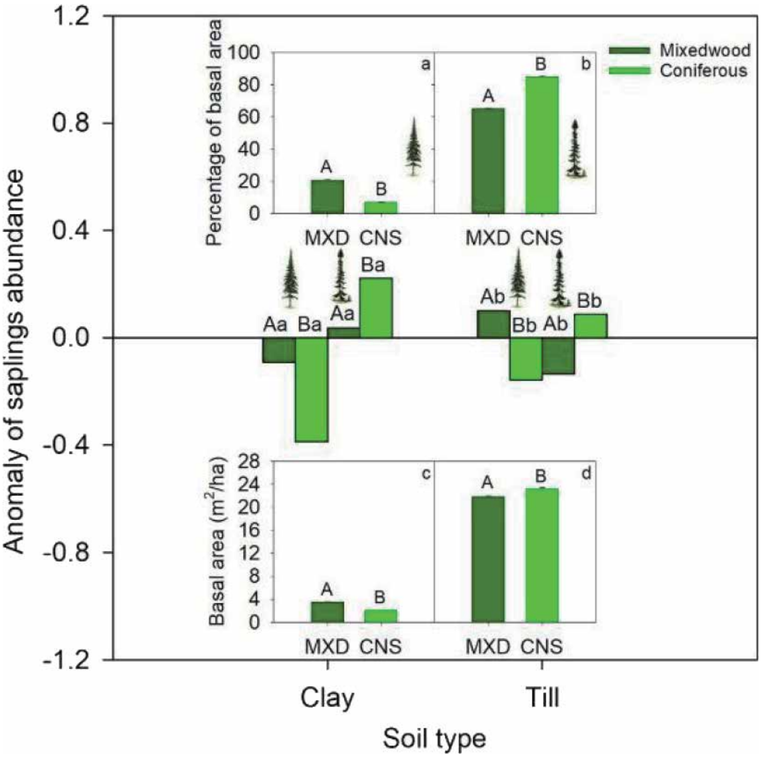

Figure 6.

Anomaly of balsam fir and black spruce sapling abundance ln(stems/ha)), according to bioclimatic domain and soil type. The anomaly represents the deviation from mean abundance of saplings for each species across the entire study area (positive = sapling excess, negative = sapling deficiency). Inset panels indicate percentage of basal area between the two bioclimatic domains (MXD = mixedwood, CNS = coniferous) for parental trees of balsam fir (a), black spruce (b), and the absolute values of the basal area for other tree species (c), and for the stand (d). The dark and light green histograms indicate the mixedwood and coniferous domains, respectively. The letters at the top of the histograms indicate nonsignificant (same letters) or significant (different letters) differences between the mixedwood and coniferous bioclimatic domains. The lowercase letters show the comparison between till and clay soils within each bioclimatic domain (modified from [26]).

research [11]. Negative influences of clay soils on balsam fir appear be related to higher water content often characterizing clay soils, having lower temperatures and lower rates of evaporation. Saplings of balsam fir growing on clay are bound to encounter oxygen deprivation in the rooting zone due to waterlogging and reduced gas exchange, conditions that are transcendently found in the coniferous domain. It has been additionally mentioned that lower temperatures in clay soils seem to promote increased organic matter accumulation, unfavorable for balsam fir establishment and survival [11, 33]. This suggests that site species composition, local climate, or soil characteristics are also factors, determining regeneration success, especially for balsam fir. In addition, the hard link between parental trees and regeneration for balsam fir on the one hand and the noticed inconsistency noted for black spruce on the other proposes that balsam fir is more dependent on parental tree proximity compared with black spruce. Contrasting large balsam fir seeds falling beneath or close to their parent tree, the small size of black spruce seeds allows them to spread further (effective distances of 20–80 m; [10, 40] from the parent tree. The basal area of other species on sites that are primarily composed of balsam fir or black spruce can be used as a proxy for forest composition, which could influence sapling abundance to some extent. In fact, basal area of other tree species dominated by deciduous species was greater in the mixedwood than in the coniferous bioclimatic domain (**Tables A2** and **A3**). The presence of deciduous species, such as paper birch or trembling aspen, two dominant deciduous species in our study area, may favor suitable conditions (e.g., higher soil temperatures, increased organic layer decomposition, and impeded paludification), hence increasing sapling survival, especially for the balsam fir [34]. Indeed, our results confirmed the higher sensibility of balsam fir regeneration to the environment condition than black spruce. In addition, large balsam fir seeds can be protected from seed predation and competition with herbaceous plants by a broadleaf litter layer, as long as it arises from the small leave trees such as birch and aspen [24, 43, 44]. In contrast, thicker broadleaf litter layers appear to be detrimental for small seeded tree species, such as black spruce, because their seeds contain fewer nutritional reserves for germination and sufficient root elongation to penetrate the mineral soil through the litter layer [45]. Thus, the lower density of other species in the coniferous domain could trigger a population shift from warmer balsam fir conditions to colder conditions to which black spruce and associated species are better adapted [10]. We would posit that a higher diversity of forest composition exerts two major effects upon the regeneration: facilitation for balsam fir and exclusion for black spruce. Yet, the effects of exclusion were linked more indirectly to the negative effects of broadleaf litterfall on black spruce regeneration, as previously mentioned.

To maintain mean regeneration, balsam fir required on clay soil at least 40% and 44% of parental trees in the mixedwood and coniferous bioclimatic domains, respectively. However, the requirement was only 27% and 37% of parental trees on till soil in the mixedwood and in the coniferous domain, respectively (**Figure 7a**). Conversely,

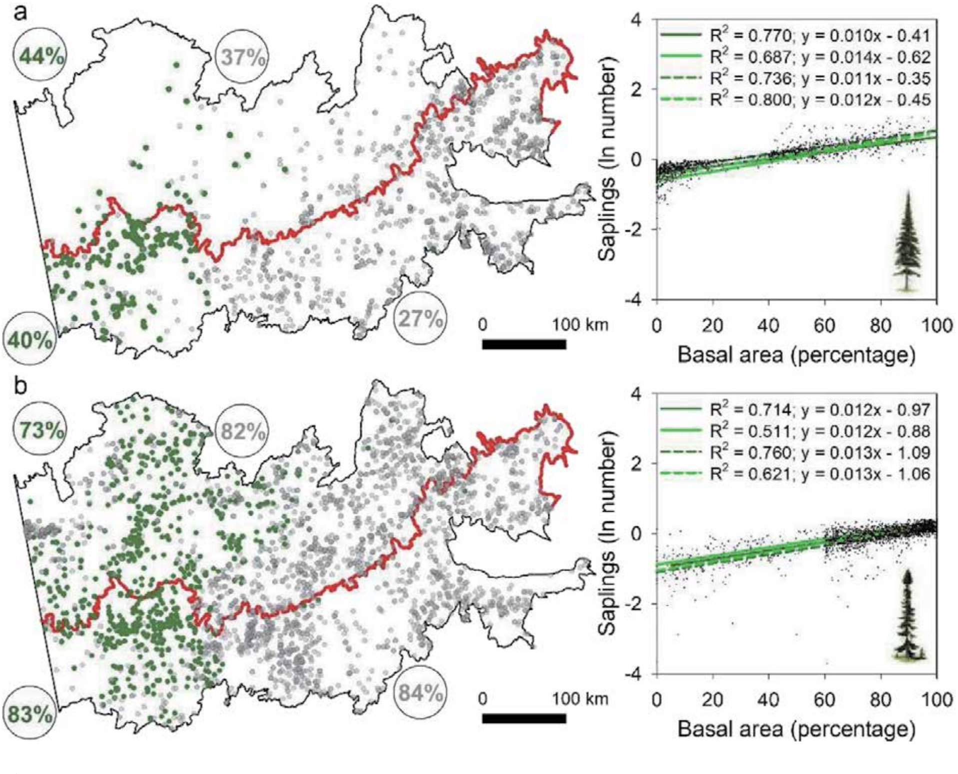

Figure 7.
Dark green and gray circled values pertain to the respective clay and till soils inside a given forest domain. Scatter plots and trend lines depict relationships between the anomaly of sapling abundance and percent basal area of parental trees. The dark green solid and dashed lines respectively indicate clay and till soils in the mixedwood domain, while the light green solid and dashed lines respectively indicate clay and till soils in the coniferous domain.

black spruce required 82–84% of parental trees to maintain mean regeneration, except on clay soils in the coniferous bioclimatic domain (73%; **Figure 7b**). This indicates that balsam fir required more parental trees (seed source) to maintain mean regeneration in coniferous than in mixedwood bioclimatic domains. This contrasts with black spruce, which requires higher numbers of parental trees than does balsam fir, although the percentage was slightly lower in coniferous bioclimatic domain, especially in clay soil. This might be explained by lower black spruce seed inputs compared with those of balsam fir, due the smaller semi-serotinous cones constituting an aerial seed bank for former, which can release small quantities of seed continuously but episodically with the occurrence of fire [9, 46]. Another explanation may be related to higher seedling mortality within the low light understory for the less shade-tolerant black spruce. Another interesting finding is that black spruce regeneration required an equivalent percentage of parental trees basal area, except on clay soil in coniferous bioclimatic domain, where the percentage was the lowest. Conversely, balsam fir regeneration required more parental trees basal area on clay than on till soils regardless of the bioclimatic domain, adding the stronger role of the substrate on the balsam fir regeneration success compared with black spruce.

The effect of the climate on regeneration showed a contrasting effect of GDD_5, with a positive and negative relationship on balsam fir and black spruce regeneration, respectively (**Table 4**), illustrating greater adaptation to warmer environments that was shown by balsam fir compared with black spruce [10]. The effect of GDD_5 was stronger and positive because clay is known to be colder, with a greater water-holding capacity than till soils [33, 47], which are not only warmer, but are subject to greater rates of moisture evaporation [48]. Although, saplings have deeper roots than seedlings, saplings are tall individual, making them less shaded by the forest canopy. This may explain the positive and the absence of the PP_MA on balsam fir saplings on till and clay soils, respectively. Another explanation is that the absence of significant effect of PP_MA on balsam fir saplings on clay soil could be due to the contrasting effect of the clay. Indeed, in mixedwood bioclimatic domain, clay soil prevents balsam fir regeneration from the water stress due to warmer temperature, while it has a negative effect in coniferous bioclimatic domain due to its lower temperature and higher amount of water, unfavorable condition for balsam fir establishment. The negative effect of GDD_5 on black spruce regeneration irrespectively of the soil type confirmed its lower tolerance to higher temperatures than balsam fir [10], which are found mostly in southern locations. More, the effect of PP_MA on black spruce regeneration was negative on till and not significant on clay soils. On clay soils, black spruce demonstrated its adaptation to cold soil temperatures and higher water content [49]. The negative relationship between PP_MA and black spruce regeneration found on till soils appears to be due to competition for water with coexisting deciduous species, since till soils are subject to greater water drainage and higher rates of evaporation. The interaction between GDD_5 and PP_MA, indicating the drought effect, showed a significant positive and negative relationship for balsam fir and black spruce

Species	Soil type	GDD_5	PP_MA	GDD_5*PP_MA
Balsam fir	Clay	**0.140****	−0.043	**0.129***
	Till	**0.091****	**0.190****	**0.189****
Black spruce	Clay	**−0.246****	0.055	**−0.221****
	Till	**−0.052***	**−0.191****	**−0.134****

*Correlations that significantly different from zero ($p < 0.05$) are shown in boldface type: *$p < 0.01$; **$p < 0.001$.*

Table 4.
Person correlations (r) between species saplings abundance and climate variables according to the soil type.

regeneration, respectively of the soil type (**Table 4**). This indicates that drought did not influence negatively on balsam fir regeneration, while it did on black spruce regeneration. Therefore, balsam fir seems to be more drought-tolerant compared with black spruce, explaining the adaptation of balsam fir to warmer conditions compared with cooler and moister conditions for black spruce [10]. Unexpectedly, the positive effect of drought on balsam fir regeneration was more obvious on till than on clay soils (r = 0.189 vs. 0.129), probably due to the higher occurrence of till soil in central and eastern parts of the study area, where precipitation increases eastward (**Figure A1**). Thus, balsam fir was less adapted to the occurrence greater soil moisture levels. Another explanation is that since black spruce is less shade-tolerant than balsam fir [10], black spruce regeneration occurs under lower forest cover and, thus, is more exposed to drought conditions [44, 50]. Although, levels of PP_MA were comparable between the two bioclimatic domains, drought seemed to arise more frequently in the warmer mixedwood than in the cooler coniferous bioclimatic domain (**Figure A1**).

The ecocline between the two bioclimatic domains of eastern North America constitutes a shift from balsam fir to black spruce dominance rather than the northern limit of balsam fir, which extends further north (54°; [7]). This clarifies the persistence of scattered balsam fir populations in the matrix of black spruce in coniferous bioclimatic domain, where reduced regeneration did not deal with the stability of such populations, just as long as a minimum parental tree basal area remained to support mean regeneration. The insight provided by the current study agrees with prior findings indicating that these few balsam fir populations can seemingly exist for a long time in the absence of severe disturbance such as fire [8]. Indeed, wildfire is a major disturbance in the boreal forest, and it can have a significant impact on the composition and dynamics of the vegetation at any particular place [21, 51]. Balsam fir is well known to be fire-intolerant species because its thin bark offers weak protection against fires [10]. Unlike black spruce, balsam fir cannot maintain a seedbank in the tree crown. Thus, its low abundance has been linked to large and intense fire regimes [10]. Furthermore, large fires may kill the parental trees of balsam fir, compromising regeneration success [52]. In contrast, black spruce is well adapted to fire because of its thick bark, which offers efficient protection against fire. Also, black spruce benefits more from large fires, owing to its aerial seed bank that persists in serotinous cones remaining in the canopy for many years [9], until a fire opens them to liberate seeds.

4. Conclusion

The ecocline between the mixedwood and coniferous bioclimatic domains of boreal zone in eastern North America represents a shift of the balsam fir and black spruce dominance below and above this ecocline. Thus, a decline of this dominance strongly impacted the regeneration success for both species, especially for balsam fir. Soil type played a different role below and above this ecocline depending on the species, the bioclimatic domain, climate, and at the lesser extend the regeneration stage (seedlings vs. saplings). However, in coniferous bioclimatic domain, clay soil (lower temperature and higher water holding), increase of black spruce, and decrease of deciduous stand tree basal area, lower air temperature affected negatively balsam fir regeneration. Unexpectedly, morality rate was higher in mixedwood than in coniferous bioclimatic domains regardless for the soil type and higher on till than on clay soils in coniferous bioclimatic domain. This highlights the importance of the competition as the regeneration density increases. In addition, mortality rate declines with increasing the basal area of other tree species in coniferous bioclimatic domain, adding the importance of deciduous species promoting balsam fir regeneration in more limiting environment. The results also confirmed the contrasting adaptation of balsam fir and black spruce to the temperature and drought conditions through their regeneration dynamic. To maintain regeneration equivalent to the mean for the entire study area, balsam fir and black spruce required, according to the soil type, respectively 4–10% more and 2–10% less parental trees in the coniferous than in the mixedwood bioclimatic domain. This is related to the lower adaptation of balsam fir to lower temperatures and shorter growing seasons in coniferous bioclimatic domain. Moreover, the parental trees basal area requirement for the black spruce regeneration success was pretty much less affected by the bioclimatic domain and soil type, except on clay soil in coniferous bioclimatic domain, where it was the most successful. Conversely, this requirement for the balsam fir success was associated to the bioclimatic domain and to the soil type, but higher on clay soil regardless of the bioclimatic domain, confirming the lower adaptation of balsam fir to the more water-holding soil compared with black spruce. Nonetheless, the threshold of parental tree basal area required for species regeneration to be equal to the mean study area is lower for balsam fir compared with black spruce, irrespectively of the bioclimatic domains, explaining the occurrence of mixedwood balsam fir populations well above the ecocline between the two bioclimatic domains. Consequently, our research confirms that this ecocline does not reflect the northern limit of balsam fir species, as shown by scattered but viable balsam fir populations found in the matrix of the coniferous forest domain, even further north of the ecocline. Another new insight given by this study is the both spatial scales (local and landscape) that were tended to, which significantly builds our comprehension of vegetation dynamics in the boreal biome inside the setting of future global change.

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Conflict of interest

There is no conflict of Interest.

Notes/thanks/other declarations

No declaration.

A. Appendix

Data	Stand	Variables	BF_BA	BS_BA	Others_BA	Total_BA
Field		BF_BA				
		BS_BA	−0.164			
		Others_BA	0.085	**0.918****		
		Total_BA	**0.508****	**0.732****	**0.875****	
Inventory	Balsam fir	BF_BA				
		BS_BA	**0.126****			
		Others_BA	**0.331****	**−0.278****		
		Total_BA	**0.873****	**0.307****	**0.620****	

Data	Stand	Variables	BF_BA	BS_BA	Others_BA	Total_BA
	Black spruce	BF_BA				
		BS_BA	**0.124****			
		Others_BA	**0.035**	**0.213****		
		Total_BA	**0.228****	**0.975****	**0.397****	

*BF_BA and BS_BA indicate the basal area for balsam fir and black spruce respectively, while Other_BA and Total_BA indicate the basal area for other tree species and the basal area of the stand respectively. In bold significant level (0.01 < α < 0.05, *0.001 > α < 0.01, **α < 0.001.*

Table A1.
Pearson correlation coefficient between the different basal areas.

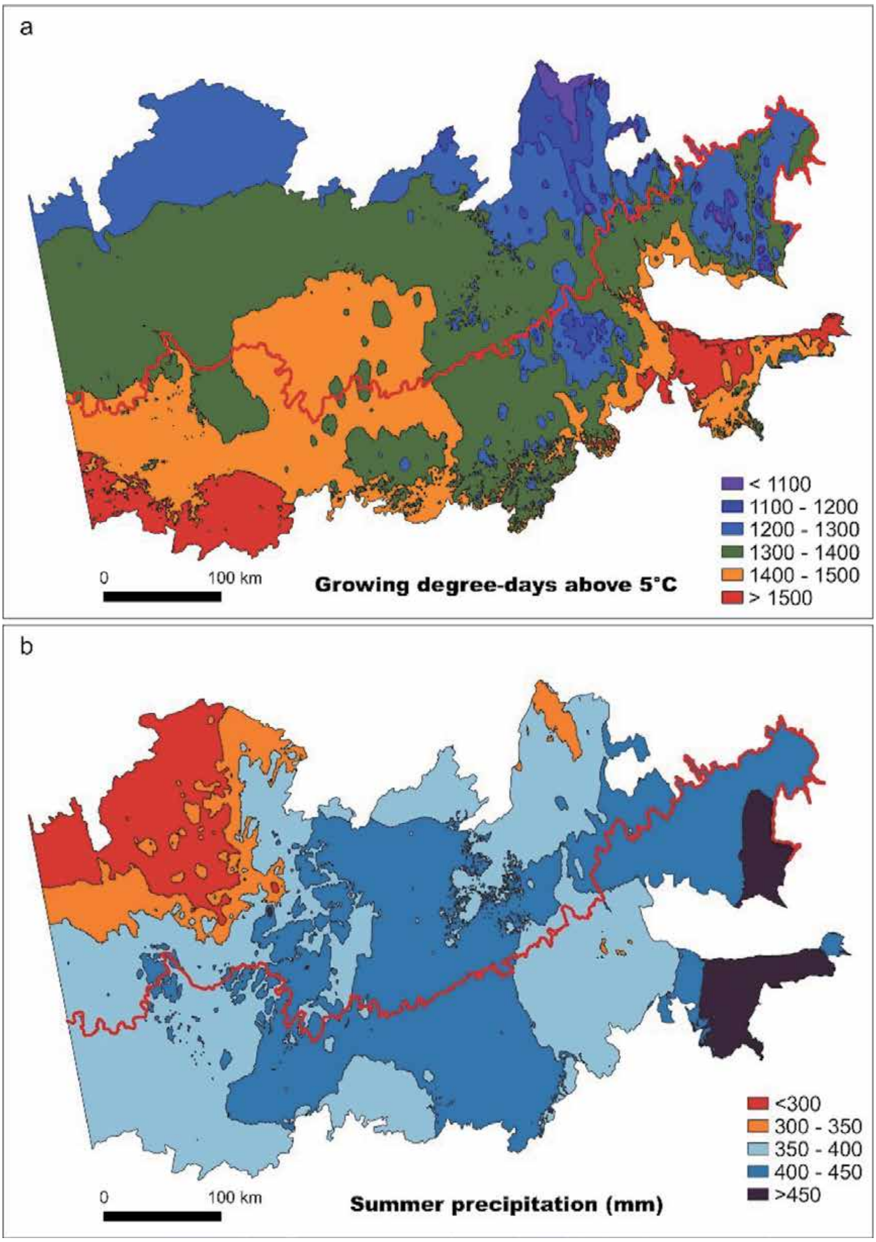

Figure A1.
Distribution of the climate variables in the study area: (a) growing degree-days; and (b) total summer precipitation (May–August). Red line indicates the boundary between the mixedwood and coniferous bioclimatic domains.

Species	Scientific name	Balsam fir stand		Black spruce stand	
		Mixedwood	Coniferous	Mixedwood	Coniferous
Deciduous	*Sorbus americana* Marsh.	0.051 (0.010)	0.006 (0.003)	0.000 (0.000)	0.000 (0.000)
	Populus balsamifera L.	0.029 (0.016)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
	Prunus serotina Ehrh.	0.004 (0.004)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
	Alnus viridis (Vill.) Lam. & DC.	0.021 (0.007)	0.024 (0.008)	0.000 (0.000)	0.000 (0.000)
	Alnus incana (L.) Moench	0.213 (0.042)	0.023 (0.013)	0.000 (0.000)	0.000 (0.000)
	Betula populifolia Marsh.	0.000 (0.000)	0.003 (0.003)	0.000 (0.000)	0.000 (0.000)
	Acer spicatum Lam.	0.020 (0.007)	0.002 (0.002)	0.000 (0.000)	0.000 (0.000)
	Sorbus decora (Sarg.) Schneid.	0.000 (0.000)	0.006 (0.003)	0.000 (0.000)	0.000 (0.000)
	Betula papyrifera Marsh.	3.932 (0.277)	2.137 (0.200)	1.178 (0.095)	0.604 (0.065)
	Prunus pensylvanica L. f.	0.089 (0.018)	0.004 (0.004)	0.000 (0.000)	0.000 (0.000)
	Acer rubrum L.	0.078 (0.024)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
	Amelanchier canadensis (L.) Medik.	0.006 (0.003)	0.002 (0.001)	0.000 (0.000)	0.000 (0.000)
	Populus tremuloides Michx.	1.025 (0.158)	0.573 (0.119)	0.505 (0.061)	0.294 (0.043)
	Salix sp.	0.166 (0.028)	0.051 (0.013)	0.085 (0.012)	0.052 (0.007)
	Betula alleghaniensis Britt.	0.092 (0.045)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
Coniferous	*Pinus banksiana* Lamb.	0.398 (0.067)	0.652 (0.171)	1.026 (0.084)	1.386 (0.094)
	Thuja occidentalis L.	0.000 (0.000)	0.010 (0.010)	0.000 (0.000)	0.000 (0.000)
	Picea rubens Sarg.	0.033 (0.020)	0.026 (0.026)	0.000 (0.000)	0.000 (0.000)
	Larix laricina (Du Roi) K. Koch	0.149 (0.037)	0.223 (0.087)	0.278 (0.040)	0.189 (0.034)
	Pinus strobus L.	0.004 (0.004)	0.000 (0.000)	0.005 (0.005)	0.000 (0.000)
	Picea glauca (Moench) Voss.	1.136 (0.145)	0.696 (0.144)	0.232 (0.039)	0.081 (0.022)

Standard errors are ncluded in parentheses.

Table A2.
Composition of canopy species other than black spruce and balsam fir in the study area and their mean basal area (m^2 ha^{-1}) in each bioclimatic domain.

Deciduous tree cover	**Soil type**	**Bioclimatic domain**	
Regeneration		**Mixedwood**	**Coniferous**
Balsam fir	Clay	74.81^{A} (3.02)	61.63^{B} (5.69)
	Till	73.14^{A} (1.48)	73.07^{A} (2.74)
	Total	***73.56^{A} (1.43)***	***70.48^{A} (2.49)***
Black spruce	Clay	55.11^{A} (3.24)	42.37^{B} (3.38)
	Till	55.99^{A} (1.97)	44.26^{B} (2.06)
	Total	***55.75^{A} (1.68)***	***43.78^{B} (1.76)***

The uppercase superscript on each mean value indicates a nonsignificant (same letter) or significant (different letters) difference between mixedwood and coniferous bioclimatic domains. Standard errors are in parentheses.

Table A3.
Average proportion of deciduous species on sites that were dominated by balsam fir or black spruce regeneration in the mixedwood and coniferous bioclimatic domains.

Author details

Yassine Messaoud
University of Quebec in Abitibi-Temiscamingue, Rouyn-Noranda, Canada

*Address all correspondence to: ymessaou@lakeheadu.ca

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Chapter 3

Research Progress on Iron-Heart *Cunninghamia lanceolate*

Ninghua Zhu, Xiaowei Yang, Zhiqiang Han and Xiao Can

Abstract

Cunninghamia lanceolate (Lambert.) Hooker is one of the main fast-growing timber forest species in southern China which has a long history of cultivation and spreads across 28 provinces, cities, and regions. Recently, a variant of fir was discovered in the Xiaoxi National Nature Reserve in Hunan Province. The heartwood is hard as iron and its ratio is more than 80%, with the especial character of anti-corruption. It is a natural germplasm resource, called Iron-heart *Cunninghamia lanceolate*. Study on it is still in the stage of data accumulation. In this paper, we studied it from three points as follows: (1) Plus tree selection and construction of germplasm resources nursery. (2) Study on cone and seed quality. (3) Genetic structure analysis of natural population. The research of Iron-heart *Cunninghamia lanceolate* lays a theoretical foundation for the protection, development, and utilization of the black-heart wood germplasm resources of Iron-heart *Cunninghamia lanceolate* in the future.

Keywords: germplasm collection, plus tree selection, seed and cone quality, genetic diversity

1. Introduction

The Chinese fir, *Cunninghamia lanceolate* (Lambert) Hooker, belongs to the *Cupressaceae* family, which is the family with the largest number of genera among Gymnospermae and includes a number of other significant species in particular, Taiwania Hayata, Cryptomeria D. Don, Glyptostrobus Endl, etc. [1, 2]. As an evergreen coniferous tree species, *C. lanceolate* is native to northern Vietnam and southern China. Because of its desirable wood properties, fast growth, and high disease resistance, *C. lanceolate* has been widely grown in China for 3000 years [3–5]. Recently, a unique natural wild variety of Chinese fir with a high ratio of heartwood and high wood quality was inadvertently found in provenance. Importantly, this Chinese fir has a high corrosion prevention property compared to other species, its wood is dark, and native people use it to make furniture, buildings, and even coffins [6, 7].

The study of cone and seed morphological characteristics of Iron-heart *Cunninghamia lanceolate* is helpful to master phenotypic diversity and formulate population protection strategies [8]. Selecting the best family for seed collection and seedling breeding has a key impact on improving the quality of Iron-heart China fir seedlings [9, 10]. Wild plants are important gene resources for breeding excellent varieties, so it is more important to study the genetic diversity and variation of wild populations [11].

For the germplasm resources of *Cunninghamia lanceolate*, the most common way is to preserve them by ex-situ conservation [12], and the establishment of germplasm collection area of *Cunninghamia lanceolate* is usually realized by grafting. Combining the conservation and application of germplasm resources in the nursery, on the one hand, the improved varieties were screened and preserved by selecting the best in the experimental area, on the other hand, the germplasm resources bank was enriched and high-quality breeding materials were provided. At present, in the field of science, the conservation and application of germplasm resources have been adopted by seed banks and gene banks in most countries, which can be summarized as "two less and one rich", with less use area, less funds, and rich germplasm resources [13]. In addition, the rapid development of modern biotechnology makes it possible to use tissue culture in vitro preservation of *Cunninghamia lanceolate*. In a word, we can take a variety of forms to achieve the preservation of Chinese fir germplasm resources, but we should consider different places, depending on the situation, choose the best way to collect and preserve high-quality resources.

Determining genetic diversity and population structure, which are important for characterizing germplasm under investigation, constitute important steps in plant breeding [14, 15]. However, due to the impact of agricultural climate change, morphological characteristics provide limited genetic information [16]. Therefore, molecular markers unaffected by environmental changes are necessary to estimate genetic diversity and population structure [17, 18]. Based on molecular markers, genetic diversity analysis, germplasm characterization and evolution studies have been possible in the last 30 years [19, 20]. Molecular markers, such as restriction fragment length polymorphism (RFLP), random-amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter-simple sequence repeat (ISSR) and simple sequence repeat, microsatellite (SSR), have previously been used to study the genetic diversity and population structure of cultivated and natural breeding populations of many conifers [21]. SSR markers, which are relatively abundant, inexpensive, and provide more informative than bi-allelic markers, have been used to detect the genetic diversity, population structure, and even genetic relationships among landraces and cultivars of *Cunninghamia lanceolate* [22–24]. Single-nucleotide polymorphisms (SNPs), as a type of third-generation molecular marker with high stability and diversity, are expensive to analyze compared with SSR and AFLP markers [25–27].

2. Research contents

2.1 Area sampled

Xiaoxi National Nature Reserve is located in Yongshun County, Western Hunan Province, at the western end of Wuling Mountain. It is located at 110°6′50″–110° 21′35″ E and 28°42′15″–28°53′55″ N. The annual average temperature is between 11 and 12°C, the frost-free period is 250 days, the annual precipitation is between 1300 and 1400 mm, the parent material of soil is sand shale, the soil fertility is high, the total forest storage is 2,223,500 cubic meters, the area has high mountains, dense forests, crisscross valleys, a wide variety of rare plants, rare birds and wild animals, with more than 1000 species of plants in 94 families. There are nearly 200 species of wild animals in the original secondary forest, including 68 species of national key protected animals such as leopard, clouded leopard, and white-necked long-tailed pheasant [28], which are rare in the world and unique in China. The only surviving evergreen broad-leaved primary secondary forest in the 13 provinces of central and southern China is protected from Quaternary glaciers.

2.2 Sampling design

The Iron-heart *Cunninghamia lanceolate* was listed and numbered. According to the principle of uniform dispersion and a random selection, 33 cones of mother trees (52 years old) were collected in the mother forest in mid-October 2019 and were brought back to Central South University of Forestry and Technology to dry naturally for later use in 10, 2019.

About 35 plus trees of Iron-heart *Cunninghamia lanceolate* were selected. Fresh cuttings are collected and used as materials for the establishment of a germplasm resource nursery. See Appendix **Table S1** for the basic information. The germplasm resource nursery is set up in the Chinese fir test demonstration forest base in Xichong Village, Majiang Town, Chaling County, Zhuzhou City, Hunan Province. It has red soil and good site conditions. The demonstration forest has Guangxi provenance seedlings (Guangxi-2.5) and Fujian provenance vegetative Line cutting seedlings-020 (Fujian-020) and Fujian clone Zhongyuan cutting seedlings-061 (Fujian-061) pure forest of young Chinese fir, and grow well.

In total, 548 Iron-heart *Cunninghamia lanceolate* from nine plots (CTY, JZW-1, JZW-2, JZW-3, LYP-1, LYP-2, LYP-3, LYP-4, and XNC) were collected, covering the entire range of Iron-heart *Cunninghamia lanceolate* from (Appendix **Table S2**) (According to the natural distribution of the natural population of iron-heart Cunn*inghamia lanceolate*, we found that it is concentrated in 9 mountains. Therefore, we divided it into 9 plots for population genetic structure analysis). Growth indexes and morphological parameters were considered as selection criteria for the sampled trees, which were chosen by a dominant comparative and comprehensive evaluation method in typical natural forests. The longitude, latitude, and altitude of each sample were determined using a handheld GPS (WGS-84) (Garmin eTrex Handheld GPS; Garmin). Fresh leaves of each voucher sample were collected in a 10 ml freezing tube, transported back to the laboratory in a liquid nitrogen tank, and deposited at −80°C.

2.3 Data sampled

2.3.1 Quality determination of cones and seeds

1. Take 10 kg fresh cones from each plant and dry them. Test the quality of cones and seeds. Repeat for 3 times in each family. The cone length, cone width, seed length, and seed width are measured with a vernier caliper. The total cone quality and seed quality are weighed with an electronic balance (accurate to 0.01 g) to calculate the cone seed extracting percentage. Cone seed extracting percentage = total seed quality ÷ total cone quality × 100%.

2. About 1000 seeds were randomly selected, and the quality of 1000 seeds (g) was measured in the air-dry state, repeated 3 times.

3. Seed goodness test. Seed goodness = real number of good seeds ÷ real number of tested seeds.

4. The seed germination rate was determined by the standard germination method. Take 150 seeds from each plant family, sterilize with 10% antifomin for 15 min, wash 3 times with sterile water, and soak in sterile water at 25°C for 24 h. Take a sterile petri dish, spread it with sterile filter paper, and moisten it with sterile water; spread the soaked seeds on the filter paper, and place them in a 25°C light incubator for cultivation. Repeat 3 times each, observe and

count the germination situation once every 5 days, and count the real number of germinated species after 15 days to calculate the germination rate (%). Germination rate = real number of germinated seeds ÷ real number of initial seeds × 100%.

2.3.2 DNA extraction, amplification, and microsatellite genotyping

A Plant Genomic DNA Kit (TIANGEN Biotech, Beijing, China) was used to extract total genomic DNA. Genomic-SSR polymerase chain reaction (PCR) was performed in a 20 μl reaction volume containing 4.0 μl double-distilled water, 4.0 μl genomic DNA, 10.0 μl 2× Taq Plus PCR MasterMix (TIANGEN, Beijing, China), 1.0 μl forward primer and 1.0 μl reverse primer. The PCR conditions included denaturation for 5 min at 95°C, 30 cycles of 30 s at 94°C, 90 s at the annealing temperature for each SSR marker in the reaction, 1 min at 72°C, and 10 min at 60°C for a final extension. In total, 15 primer pairs with highly polymorphic loci (**Table 1**), for which the clarity and reproducibility of the DNA fragments were amplified, were selected from published papers [29–31].

The forward primer had a universal M13 primer tail and a universal M13 primer fluorescently labeled with 6-carboxy-x-rhodamine, tetramethyl-6-carboxyrhodamine, 6-carboxy-fluorescein, or 5-hexachlorofluorescein. The final PCR products were separated based on capillary electrophoresis fluorescence using an ABI3730xl DNA Analyzer (Genewiz Inc., Beijing, China). The results were analyzed using GeneMarker 1.75 software (SoftGenetics LLC, State College, PA, USA).

2.4 Statistical analyses

Excel 2019 and R4.0.3, Rstudio software were used for summary processing and nested analysis of variance, Pearson correlation analysis, and principal component analysis. Among them, R4.0.3 calculates the mean value, standard deviation, and coefficient of variation of the seed and cone traits; based on the nest's linear model variance analysis between and within groups differences, and Tukey HSD test; using R package Hmisc 4.4.2 [32] to calculate Pearson Correlation coefficient and p-value, use corrplot 0.84 [33] to draw the correlation graph.

The polymorphism information content (PIC) was used to estimate the allelic variation of SSR by applying the formula PIC = $1\text{-}\sum_{i=0}^{n} Pi^2$, where Pi is the frequency of the ith allele and n is the number of alleles detected for given SSR markers. GenALEx 6.5 [34] was used to estimate the genetic diversity indices of each locus and population.

The genetic diversity and population structure of the accessions were further investigated by analysis of molecular variance (AMOVA) using GenAlEx 6.5. The program STRUCTURE v2.3.4 [35] was used to analyze the genetic structure by employing Bayesian clustering analysis with the admixture model of independent allele frequencies. STRUCTURE HARVESTER (http://taylor0.biology.ucla.edu/structureHarvester/) was used to evaluate the most likely number (K) of genetic clusters. The data derived from the STRUCTURE analysis were visualized as bar charts and pie charts using ArcMap v10.0 and DISTRUCT v1.1 software [36, 37]. Interpolation of ArcGIS was used to forecast the expected heterozygosity (He) and the private allele frequency (Fp) of all Chinese firs included. The ArcGIS (Esri) program was used to map the distribution of the He of populations and Fp by employing a kriging spherical interpolation method.

Loci	Forward primer	Reverse primer	Repetitive unit	Product size (bp)	Ta°C
contig476_526D	TTTGGGACCTTATGGAGGTGGAG	AAACCACCAGGTTGAGAAGCAGC	(GGA)9	153–159	57.10
contig7616_683B	GAGCCGTGAAGAACGAAGGTCTC	ACGATCGGATTGTCTCAGAAACG	(GAA)12	260–281	57.05
contig4728_384B	ATTATCCGAGGCAGATACGCAC	CTTCTCCGTATTTGATCCATCGC	(GGA)10	336–354	55.05
contig5410_1886A	GGCTCGAGTTTGCATCTCACAC	CACATCCAATCCATACAGGAGGG	(TC)9	210–320	56.70
contig16181_1285C	GGTACTGCGAATCTTCAAATCC	TGTTCAAGAAAGGAAGCAAACGG	(TC)9	293–297	53.25
contig406_1209C	TCATCAGCCTCAGTTTGTACTTGC	GCAATCATGGGCTCTCTGCAC	(AT)9	348–384	56.00
Unigene685	CCTTTCTTTTCTGCACCAGC	CTGTGCCTGATGGCTAAACA	(GGT)5	190–284	56.90
Unigene754	AGACGGTCGTTGACGAAAAA	CTCTTTTCCACACACGCAAA	(GCA)4	124–298	55.35
Unigene840	CAGGACGCCTGAGAATTGTT	TCATCGGTAGAAGGAATGGC	(AAG)5	162–169	56.65
Unigene1061	GAAACAAACAAGGGAGGCAA	AGGTCCAAATCCACCTGGTC	(AGG)9	150–276	57.70
Unigene491	TGGAAATGGCTGTAAAGGAG	TGTGCTGAGCCATATTCACA	(GAAG)3	120–168	55.30
contig6319_250C	GCGGCCATTTATATCATCTTC	CACGCCTGTAATTCATCTCCGTC	(GAA)9	126–135	57.30
contig1560_1789D	TTTCGGCTCTCCGACTCCTTAAC	AGAATCGCGTCCAGAACACAGAG	(CT)11	129–147	59.45
CLSSR6	ATTTCAAACACCTCTCCTTTC	GGAATTCCTAGACAAAGATGG	(CTTC)4	136–268	52.35
CLSSR8	ATCGTTGCTTTCAATCTTATG	ATCCAACTGACACACAAAATC	(CTTT)3	143–165	51.80

Ta°C represent annealing temperature of PCR cycles

Table 1.
Primary simple sequence repeat primers used in the study.

3. Results

3.1 Cone and seed quality

In this study, we use 12 traits (germination rate, seed quality, seed length-width ratio, seed length, cone seed extracting percentage, seed width, total cone quality, goodness, cone length, Seed quality (1000), cone length-to-width ratio, cone width) to assess the of quality of cones and of seeds.

3.1.1 Differences in seed quality of different families

The results in **Table 2** (code is the number of different mother trees) show that the variation range of the cone length of Iron-heart *Cunninghamia lanceolate* is 3.15–6.13 cm, the average is 4.66 cm, and the coefficient of variation is 18.97%; the variation range of cone width is 3.56–2.15 cm, the coefficient of variation is 12.18%, and the average is 2.95 cm; the variation range of the total quality of the cone is 1.15–2.40 kg, with an average value of 1.66 kg. The variation range of seed quality is large, between 0.09–0.33 kg, the coefficient of variation is 36.50%, and the average value is 0.20 kg; the cone seed extracting percentage is 5.59–19.02%, and the coefficient of variation is 24.42%, but the overall cone seed extracting percentage is low. Seed quality of Iron-heart *Cunninghamia lanceolate* from different families is quite different. The largest seed length, seed width, and seed length-to-width ratio are 9.77 mm, 5.75 mm, and 3.03 respectively; the smallest ones are 4.36 mm, 2.05 mm, and 1.16 mm, respectively; the average value are 6.31 mm, 2.35 mm, and 2.69 mm; the coefficients of variation are 26.02%, 22.15%, and 26.91%, respectively. The average seed quality of 1000 seeds is 7.06 g, the maximum is 10.54 g; the average of goodness is 67.65%, and the coefficient of variation is 20.53%. The variation range of seed germination rate is 5.33% ~ 63.00%, the coefficient of variation is 52.34%; the seed germination rate of TXS-256 and TXS-234 families is the highest, TXS-29 and TXS -30 is the next; TXS-205, TXS-265, TXS-16 germination rates are all lower than 10%. The quality of cones and seeds of families is different in different traits, so it is impossible to evaluate the quality of cones and seeds from a single character.

The coefficient of variation is the comprehensive performance of the discrete characteristics of phenotypic traits. The greater the coefficient of variation, the greater the degree of dispersion of traits. The coefficient of variation of seed traits of 33 families is between 12.18% and 51.34%, and the coefficient of variation of each trait has a certain difference. From large to small, it is germination rate > seed quality >seed length-width ratio > seed length > cone seed extracting percentage > seed width > total cone quality> goodness> cone length > seed quality(1000) > cone length-to-width ratio > cone width (**Tables 2** and **3**).

The P value associated with total cone quality, seed quality, seed germination rate, seed goodness, seed quality (1000), seed width, and cone length-width ratio was less than 0.001 (see the **Table 4**, variance analysis of 33 iron-heart *Cunninghamia lanceolate*), indicating that these traits varied greatly among families; the P value associated with of the cone-length factor is less than 0.01, and the P values associated with other factors of other characteristics were less than 0.1. There are minor differences, and differences mainly exist between individuals. The F value of 12 seed characteristics varies from 0.757 to 965.1 between families, and the order of size is cone seed extracting percentage (0.757) < cone width (1.591) < seed length to width ratio (1.704) < seed length (1.91) < germination rate (2.87) < cone length (2.885) < seed

Code	Cone length (cm)	Cone width (cm)	Length/ width	Cone quality (kg)	Seed quality (kg)	Cone seed extracting percentage (%)
TXS-16	3.29 ± 0.03	2.66 ± 0.02	1.24 ± 0.01	1.34 ± 0.01	0.12 ± 0.02	9.22 ± 1.34
TXS-17	4.08 ± 0.05	2.54 ± 0.02	1.60 ± 0.03	1.93 ± 0.07	0.22 ± 0.02	11.25 ± 1.43
TXS-18	3.77 ± 0.02	3.07 ± 0.03	1.23 ± 0.03	1.32 ± 0.08	0.14 ± 0.01	10.63 ± 0.62
TXS-19	5.49 ± 0.02	3.09 ± 0.01	1.77 ± 0.03	1.86 ± 0.03	0.21 ± 0.01	11.12 ± 0.52
TXS-29	5.76 ± 0.03	3.17 ± 0.02	1.82 ± 0.02	2.21 ± 0.02	0.32 ± 0.05	14.33 ± 0.13
TXS-30	5.76 ± 0.03	3.17 ± 0.02	1.82 ± 0.02	2.21 ± 0.02	0.32 ± 0.08	14.33 ± 0.13
TXS-202	4.12 ± 0.02	2.36 ± 0.01	1.74 ± 0.01	1.56 ± 0.01	0.17 ± 0.01	10.89 ± 0.55
TXS-205	3.15 ± 0.00	2.15 ± 0.03	1.46 ± 0.02	1.55 ± 0.01	0.09 ± 0.08	5.59 ± 0.31
TXS-217	5.24 ± 0.01	2.98 ± 0.05	1.76 ± 0.01	1.61 ± 0.01	0.21 ± 0.07	13.04 ± 0.07
TXS-219	5.95 ± 0.04	3.25 ± 0.01	1.83 ± 0.02	1.75 ± 0.04	0.33 ± 0.01	19.02 ± 0.74
TXS-224	4.57 ± 0.05	3.46 ± 0.01	1.32 ± 0.04	1.22 ± 0.06	0.19 ± 0.08	15.34 ± 0.42
TXS-228	5.85 ± 0.04	2.97 ± 0.01	1.97 ± 0.01	1.93 ± 0.05	0.25 ± 0.03	13.08 ± 1.19
TXS-234	5.84 ± 0.03	3.09 ± 0.06	1.89 ± 0.05	2.16 ± 0.04	0.33 ± 0.01	15.42 ± 0.84
TXS-236	5.02 ± 0.02	3.56 ± 0.02	1.41 ± 0.01	1.81 ± 0.02	0.22 ± 0.04	11.95 ± 0.31
TXS-237	3.66 ± 0.01	2.88 ± 0.01	1.27 ± 0.01	1.45 ± 0.01	0.15 ± 0.01	10.12 ± 0.37
TXS-238	4.61 ± 0.01	3.18 ± 0.01	1.45 ± 0.02	1.77 ± 0.01	0.19 ± 0.01	10.71 ± 0.39
TXS-239	4.35 ± 0.03	2.47 ± 0.01	1.76 ± 0.01	1.15 ± 0.01	0.15 ± 0.09	12.76 ± 0.34
TXS-256	5.89 ± 0.01	3.56 ± 0.00	1.66 ± 0.01	2.40 ± 0.01	0.31 ± 0.02	13.00 ± 0.76
TXS-259	5.64 ± 0.02	3.24 ± 0.04	1.74 ± 0.01	1.56 ± 0.01	0.28 ± 0.01	17.74 ± 0.38
TXS-264	3.27 ± 0.01	2.35 ± 0.01	1.39 ± 0.06	1.18 ± 0.01	0.09 ± 0.07	7.34 ± 0.36
TXS-265	3.45 ± 0.03	2.79 ± 0.03	1.24 ± 0.02	1.16 ± 0.01	0.12 ± 0.07	10.06 ± 0.35
TXS-267	3.89 ± 0.02	2.89 ± 0.05	1.35 ± 0.04	1.34 ± 0.01	0.16 ± 0.06	11.94 ± 0.07
TXS-268	5.10 ± 0.02	3.16 ± 0.01	1.62 ± 0.01	2.14 ± 0.01	0.25 ± 0.04	11.53 ± 1.74
TXS-270	4.03 ± 0.02	2.91 ± 0.02	1.39 ± 0.01	1.39 ± 0.04	0.13 ± 0.09	9.38 ± 0.03
TXS-276	3.91 ± 0.01	2.69 ± 0.02	1.45 ± 0.01	1.57 ± 0.05	0.13 ± 0.02	8.05 ± 0.29
TXS-349	4.99 ± 0.00	2.98 ± 0.01	1.67 ± 0.00	1.84 ± 0.03	0.23 ± 0.02	12.32 ± 1.29
TXS-363	3.88 ± 0.00	2.34 ± 0.02	1.66 ± 0.02	1.62 ± 0.01	0.17 ± 0.01	10.29 ± 0.25
TXS-365	5.03 ± 0.01	2.92 ± 0.45	1.77 ± 0.03	1.18 ± 0.01	0.18 ± 0.05	15.54 ± 0.5
TXS-366	3.94 ± 0.06	2.68 ± 0.01	1.47 ± 0.03	1.24 ± 0.04	0.13 ± 0.02	10.17 ± 1.58
TXS-370	4.87 ± 0.02	3.12 ± 0.01	1.56 ± 0.01	1.64 ± 0.03	0.18 ± 0.01	11.22 ± 1.00
TXS-400	6.03 ± 0.11	3.23 ± 0.01	1.87 ± 0.03	2.40 ± 0.16	0.27 ± 0.02	11.40 ± 0.37
TXS-578	4.64 ± 0.02	3.06 ± 0.02	1.52 ± 0.01	1.67 ± 0.04	0.19 ± 0.01	11.58 ± 0.75
TXS-666	4.75 ± 0.03	3.06 ± 0.01	1.55 ± 0.01	1.94 ± 0.02	0.21 ± 0.01	10.80 ± 0.36
CV/%	18.97	12.18	13.59	21.62	36.50	24.42
SD	0.88	0.36	0.21	0.36	0.07	0.03
Range	3.00	1.48	0.98	1.42	0.3	0.14
Mean	4.66	2.95	1.58	1.66	0.2	11.91

Table 2.
Cone characteristics (average ± standard deviation value) of different iron-heart Cunninghamia lanceolate.

code	Seed length (mm)	Seed width (mm)	Length/ width	Seed quality (1000) (g)	Goodness (%)	Germination rate (%)
TXS-16	4.36 ± 0.04	3.75 ± 0.01	1.16 ± 0.01	7.65 ± 0.05	76.67 ± 1.25	8.67 ± 1.25
TXS-17	6.11 ± 0.50	4.90 ± 0.09	1.25 ± 0.08	8.57 ± 0.01	77.67 ± 2.36	24.67 ± 2.05
TXS-18	5.41 ± 0.03	3.46 ± 0.01	1.56 ± 0.05	6.47 ± 0.03	62.67 ± 0.47	18.67 ± 0.47
TXS-19	7.86 ± 0.08	3.33 ± 0.03	2.36 ± 0.03	6.82 ± 0.01	73.33 ± 3.68	45.67 ± 4.11
TXS-29	9.77 ± 0.02	3.78 ± 0.03	2.59 ± 0.02	7.81 ± 0.02	75.67 ± 3.4	61.33 ± 1.25
TXS-30	9.77 ± 0.02	3.78 ± 0.03	2.59 ± 0.02	7.81 ± 0.03	75.67 ± 3.4	61.33 ± 1.25
TXS-202	4.41 ± 0.03	3.56 ± 0.01	1.24 ± 0.01	6.12 ± 0.05	48.67 ± 0.47	14.33 ± 0.47
TXS-205	4.44 ± 0.01	2.53 ± 0.03	1.76 ± 0.02	5.67 ± 0.07	47.33 ± 4.11	5.33 ± 0.47
TXS-217	8.09 ± 0.04	3.24 ± 0.01	2.50 ± 0.01	6.75 ± 0.02	74.33 ± 3.09	50.33 ± 0.47
TXS-219	9.47 ± 0.06	4.34 ± 0.01	2.18 ± 0.01	8.1 ± 0.02	75.67 ± 1.25	57.67 ± 2.62
TXS-224	6.49 ± 0.02	3.12 ± 0.05	2.08 ± 0.01	6.68 ± 0.05	69.33 ± 1.25	35.00 ± 0.82
TXS-228	8.42 ± 0.02	3.47 ± 0.01	2.43 ± 0.01	6.85 ± 0.03	76.67 ± 3.68	56.00 ± 2.16
TXS-234	9.43 ± 0.02	3.67 ± 0.02	2.57 ± 0.01	7.24 ± 0.03	75.00 ± 4.97	63.00 ± 2.83
TXS-236	6.45 ± 0.08	3.56 ± 0.03	1.81 ± 0.02	6.60 ± 0.02	66.33 ± 1.89	41.00 ± 0.82
TXS-237	5.39 ± 0.02	2.05 ± 0.06	2.63 ± 0.08	4.91 ± 0.05	42.67 ± 0.47	16.00 ± 0.01
TXS-238	6.31 ± 0.03	2.35 ± 0.01	2.69 ± 0.01	4.83 ± 0.07	33.67 ± 0.94	38.00 ± 0.82
TXS-239	5.35 ± 0.02	3.25 ± 0.04	1.64 ± 0.07	6.61 ± 0.05	72.00 ± 0.82	18.33 ± 0.47
TXS-256	9.68 ± 0.02	4.87 ± 0.03	1.99 ± 0.02	8.83 ± 0.01	81.00 ± 1.41	63.00 ± 5.10
TXS-259	8.74 ± 0.10	2.89 ± 0.01	3.03 ± 0.03	6.11 ± 0.05	47.33 ± 0.47	54.33 ± 0.47
TXS-264	4.60 ± 0.07	3.67 ± 0.06	1.25 ± 0.02	6.40 ± 0.03	63.33 ± 0.47	10.67 ± 5.91
TXS-265	4.74 ± 0.05	3.08 ± 0.01	1.54 ± 0.02	6.15 ± 0.03	53.33 ± 0.47	8.67 ± 1.25
TXS-267	6.06 ± 0.04	3.25 ± 0.01	1.86 ± 0.01	6.53 ± 0.05	64.67 ± 0.47	25.67 ± 0.47
TXS-268	6.21 ± 0.02	4.84 ± 0.06	1.28 ± 0.02	8.72 ± 0.01	83.33 ± 3.68	44.67 ± 3.09
TXS-270	5.02 ± 0.05	2.98 ± 0.05	1.68 ± 0.01	6.32 ± 0.05	58.67 ± 0.47	18.00 ± 0.82
TXS-276	4.78 ± 0.01	2.77 ± 0.02	1.73 ± 0.02	5.94 ± 0.04	51.33 ± 0.47	13.33 ± 0.47
TXS-349	6.77 ± 0.01	4.33 ± 0.01	1.56 ± 0.00	8.32 ± 0.01	81.00 ± 2.94	34.00 ± 2.16
TXS-363	4.88 ± 0.02	3.04 ± 0.04	1.60 ± 0.02	5.35 ± 0.05	50.00 ± 0.82	28.67 ± 0.47
TXS-365	6.23 ± 0.02	3.56 ± 0.03	1.75 ± 0.01	6.63 ± 0.05	66.00 ± 0.82	31.67 ± 0.47
TXS-366	5.74 ± 0.18	3.56 ± 0.05	1.62 ± 0.07	7.13 ± 0.05	74.00 ± 3.56	28.67 ± 0.47
TXS-370	6.36 ± 0.02	4.88 ± 0.00	1.30 ± 0.00	8.21 ± 0.00	81.33 ± 3.30	35.33 ± 0.94
TXS-400	8.83 ± 0.04	5.75 ± 0.04	1.54 ± 0.01	10.54 ± 0.02	87.67 ± 1.89	59.33 ± 1.25
TXS-578	6.67 ± 0.02	4.68 ± 0.02	1.43 ± 0.05	8.31 ± 0.01	85.33 ± 2.05	38.00 ± 2.45
TXS-666	5.96 ± 0.04	3.80 ± 0.02	1.57 ± 0.02	7.76 ± 0.04	78.00 ± 2.16	35.33 ± 0.47
CV/%	26.02	22.15	26.91	17.23	20.53	52.34
SD	1.72	0.81	0.5	1.22	0.14	0.18
Range	5.48	3.78	1.92	5.74	0.56	0.63
Mean	6.62	3.64	1.87	7.06	67.65%	34.80%

Table 3.
Seed characteristics (average ± standard deviation value) of different iron-heart Cunninghamia lanceolate.

Source of variation	Df	SS	MS	F
Total cone quality	61	8078	132.42	5.454 ***
Total seed quality	26	4827	185.64	3.221 ***
Cone seed extracting percentage	87	7692	88.41	0.757
Germination rate	45	6364	141.42	2.87 ***
Goodness	39	8408	215.59	22.39 ***
Seed quality(1000)	93	8976	96.51	965.1 ***
Seed width	72	8762	121.7	14.8 ***
Seed length	80	8030	100.38	1.91.
Length/width(seed)	68	7130	104.85	1.704.
Cone length	70	7883	112.61	2.885 **
Cone width	59	6341	107.47	1.591.
Length/width(cone)	53	7353	138.73	3.845 ***

*Note: "***": P < 0.001; "**": P < 0.01; "*": P < 0.05; ".": P < 0.1; "": P < 1.*

Table 4.
Variance analysis of 33 iron-heart Cunninghamia lanceolate*.

quality (3.221) < cone length-to-width ratio (3.845) < total cone quality (5.454) < seed width (14.8) < goodness (22.39) < seed quality (1000, 965.1).

3.1.2 Correlation analysis of seed traits of iron-heart Cunninghamia lanceolate

It can be seen from the **Figure 1** that the seed germination rate of iron-heart *Cunninghamia lanceolate* is positively correlated with the other 8 characteristics except for the total cone quality, seed quality, and cone seed extracting percentage. Among them, the germination rate is positively correlated with the cone length, seed quality, seed length-to-width ratio, and seed length are extremely significantly positively correlated at the level of P < 0.001, and are more correlated with seed width, seed length-to-width ratio, seed quality (1000), and goodness at P < 0.01; There was a very significant negative correlation (r = −0.56, P < 0.001) between cone seed extracting percentage and total cone quality, and a very significant

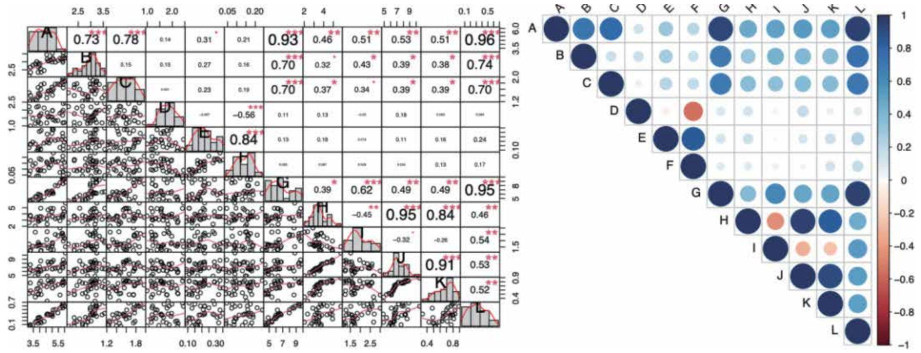

Figure 1.
Correlation analysis of seed and cone characters of iron-heart Cunninghamia lanceolate*. Note: A–L are: cone length, cone width, cone length-width ratio, total cone quality, seed quality, cone seed extracting percentage, seed length, seed width, seed length-width ratio, seed quality (1000), goodness, and germination rate.*

positive correlation (r = 0.84, P < 0.001) with seed quality, and the correlation between these three traits and the other eight traits was not significant.

3.1.3 Comprehensive evaluation of seed quality of iron-heart Cunninghamia lanceolate

Analyzing the various characteristics that affect the quality of the cones and seeds of the Iron-heart *Cunninghamia lanceolate*, it can be seen from the figure that principal components 1 and 2 can explain 65.8% of the variation (**Figure 2**). Among them, traits A, B, C, G, L has a greater contribution rate to principal component 1, and most of them are cone traits; traits H, J, K, I has a large contribution rate to principal component 2, and most of them are seed traits. The principal component dimensionality reduction method is used to comprehensively evaluate the 12 cones and seed traits of iron-heart *Cunninghamia lanceolate*. It can be seen from the **Table 5** that the cumulative variance contribution rate of the first three main factors can reach 82.30%, which can satisfy the traits of each half-sibling progeny. Therefore, the first three main factors are selected to make a comprehensive evaluation score for iron-heart *Cunninghamia lanceolate*. Take the characteristic value of the main factor as the weight of each index, and multiply each index to obtain the calculation formula of the main factor comprehensive evaluation score:

$$\begin{aligned} F_1 = {} & (0.408X_1 + 0.308X_2 + 0.308X_3 + 0.145X_5 + 0.106X_6 + 0.389X_7 \\ & + 0.286X_8 + 0.135X_9 + 0.315X_{10} + 0.310X_{11} + 0.403X_{12}) \times \sqrt{2.334} \end{aligned} \tag{1}$$

$$\begin{aligned} F_2 = {} & (0.156X_1 + 0.142X_2 + 0.104X_3 - 0.124X_4 + 0.130X_6 + 0.191X_7 \\ & - 0.453X_8 + 0.573X_9 - 0.417X_{10} - 0.369X_{11} + 0.160X_{12}) \times \sqrt{1.564} \end{aligned} \tag{2}$$

$$F_3 = (0.441X_4 - 0.541X_5 - 0.668X_6 + 0.152X_7 + 0.161X_9) \times \sqrt{1.408} \tag{3}$$

The variance contribution rates of the first three main factors are different. In the comprehensive evaluation of growth traits, the focus of each factor needs to be coordinated. The contribution rates of the three factors are 45.4%, 20.4%, and 16.5% as weights, combined with 3 common factors. The contribution rate and

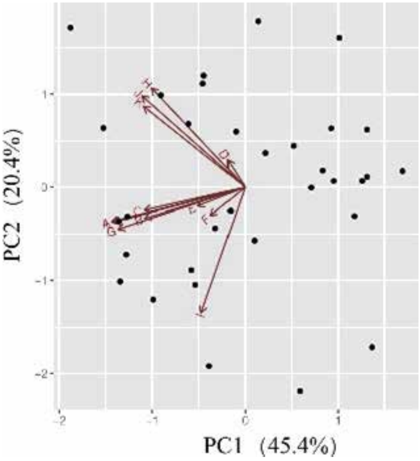

Figure 2.
PCA analysis. Note: A–L are: cone length, cone width, cone length-width ratio, total cone quality, seed quality, cone seed extracting percentage, seed length, seed width, seed length-width ratio, seed quality (1000), goodness and germination rate.

factor score Fi, refer to the calculation formula of the comprehensive score, the mathematical model of the comprehensive score of seed traits of iron-heart *Cunninghamia lanceolate* can be established:

$$D_n = F_1 \times 45.4\% + F_2 \times 20.4\% + F_3 \times 16.5\% \tag{4}$$

Using the comprehensive ranking as an indicator, a total of 14 excellent Iron-heart Cunninghamia lanceolate were selected with a 40% selection rate (**Table 6**).

3.2 Seed garden construction

3.2.1 Grafting and management of the germplasm resource nursery of Iron-heart Cunninghamia lanceolate

3.2.1.1 Seedling grafting

Before grafting, we selected high-quality rootstocks to mark and hang tags. The height of the rootstocks was uniformly about 15.6 cm. The rows are 2 m × 2 m, and at least 10 plants should be planted for each clone. After grafting, apply an appropriate amount of organic fertilizer according to the standard of 30–60 t per hectare to promote the growth and development of Iron-heart *Cunninghamia lanceolate* and improve the survival rate, stress resistance, cold resistance, and adaptability of grafted seedlings. The trails are set up in Iron-heart *Cunninghamia lanceolate* germplasm resource nursery, which is mainly used for convenient work such as planting, cultivation, observation, management, and protection. At present, 35 genotypes of superior trees selected from nature reserves are still preserved in the germplasm resource nursery (**Table** 7). In May of the same year, the research team conducted statistics and surveys on the survival rate of grafting.

Traits	**Comp.1**	**Comp.2**	**Comp.3**	**Comp.4**	**Comp.5**
A	0.408	0.156			
B	0.308	0.142		−0.456	−0.511
C	0.308	0.104		0.407	0.598
D		−0.124	0.441	−0.622	0.486
E	0.145		−0.541	−0.459	0.313
F	0.106	0.130	−0.668		
G	0.389	0.191	0.152	0.105	
H	0.286	−0.453			
I	0.135	0.573	0.161		
J	0.315	−0.417			
K	0.310	−0.369			−0.135
L	0.403	0.160			
λ	2.334	1.564	1.408	0.972	0.896
Contribution rate	0.454	0.204	0.165	0.079	0.067
Total contribution rate	0.454	0.658	0.823	0.902	0.969

Table 5.
PCA analysis of iron-heart Cunninghamia lanceolate.

Code	F1	F2	F3	Score	Ranking
TXS-16	13.878	−3.074	1.581	5.934	28
TXS-17	15.059	−4.192	2.067	6.323	22
TXS-18	13.916	−1.64	1.8	6.28	23
TXS-19	15.962	−0.623	2.64	7.555	10
TXS-29	17.937	−0.83	3.098	8.485	3
TXS-30	17.846	−1.248	2.976	8.339	4
TXS-202	12.952	−2.023	1.646	5.739	32
TXS-205	12.394	−0.859	1.517	5.702	33
TXS-217	15.963	−0.385	2.523	7.585	9
TXS-219	18.056	−1.591	2.678	8.315	5
TXS-224	14.879	−0.851	1.957	6.904	16
TXS-228	16.393	−0.56	2.721	7.777	8
TXS-234	17.425	−0.59	3.006	8.286	6
TXS-236	15.077	−1.269	2.257	6.959	14
TXS-237	12.626	0.83	2.081	6.245	24
TXS-238	13.447	1.004	2.373	6.701	19
TXS-239	13.838	−1.612	1.685	6.232	25
TXS-256	19.568	−2.255	3.082	8.932	1
TXS-259	15.982	0.817	2.626	7.856	7
TXS-264	13.25	−2.174	1.579	5.833	31
TXS-265	13.06	−1.388	1.613	5.912	30
TXS-267	14.314	−1.195	1.943	6.575	20
TXS-268	16.472	−3.745	2.226	7.082	13
TXS-270	13.361	−1.325	1.788	6.091	26
TXS-276	12.877	−1.063	1.86	5.936	27
TXS-349	16.23	−2.921	2.282	7.149	11
TXS-363	12.779	−0.921	1.844	5.918	29
TXS-365	14.714	−1.365	1.844	6.706	18
TXS-366	14.65	−1.933	1.859	6.563	21
TXS-370	16.1	−3.405	2.052	6.953	15
TXS-400	20.197	−4.234	2.879	8.781	2
TXS-578	16.309	−3.182	2.141	7.109	12
TXS-666	15.245	−2.475	2.172	6.775	17

Table 6.
Comprehensive score and ranking of principal components of 33 black-heart wood Chinese fir.

3.2.1.2 Statistics of graft survival rate

The construction of iron-heart *Cunninghamia lanceolate* germplasm resource nursery was uniformly carried out by splitting, and from the results (**Table 8**), the average survival rate of grafting was 83%, among which the minimum survival rate of grafting with number TXS-35 was 50%, and the number TXS-30, the highest

Code	Breast diameter/cm	Altitude/m	GPS(E,N)
TXS-1	72.0	895	110.246958, 28.835133
TXS-2	48.2	899	110.247068, 28.835290
TXS-3	41.1	845	110.247144, 28.833239
TXS-4	43.2	505	110.260574, 28.814858
TXS-5	29.5	560	110.260804, 28.814984
TXS-6	44.1	563	110.260804, 28.814984
TXS-7	63.0	813	110.246442, 28.822674
TXS-8	25.3	804	110.246186, 28.822969
TXS-9	51.5	800	110.247032, 28.823265
TXS-10	47.0	807	110.247682, 28.823303
TXS-11	43.0	894	110.245943, 28.834792
TXS-12	35.5	1000	110.242849, 28.834510
TXS-13	37.4	1010	110.243119, 28.835489
TXS-14	47.0	904	110.245980, 28.835239
TXS-15	46.0	894	110.246676, 28.834436
TXS-16	43.2	648	110.268425, 28.798110
TXS-17	26.9	632	110.268312, 28.797980
TXS-18	27.7	648	110.268386, 28.798233
TXS-19	40.6	632	110.267831, 28.797993
TXS-20	34.4	629	110.267767, 28.796707
TXS-21	54.7	902	110.247119, 28.835550
TXS-22	54.2	901	110.246885, 28.835296
TXS-23	73.0	895	110.246958, 28.835133
TXS-24	48.2	899	110.247968, 28.835290
TXS-25	41.1	845	110.247444, 28.833239
TXS-26	43.2	505	110.260574, 28.814858
TXS-27	29.5	560	110.260804, 28.814984
TXS-28	52.3	490	110.261149, 28.814843
TXS-29	38.7	514	110.261320, 28.814457
TXS-30	27.3	511	110.261126, 28.814625
TXS-31	29.4	524	110.260469, 28.814065
TXS-32	29.0	502	110.259730, 28.813559
TXS-33	37.7	494	110.269097, 28.814165
TXS-34	28.0	525	110.259179, 28.814850
TXS-35	31.8	523	110.250914, 28.814820

Table 7.
The information of the 35 Iron-heart Cunninghamia lanceolate.

survival rate of grafting is 96%. It shows that TXS-30 has a high degree of adherence to the test forest fir, and it is suitable as a material for remote preservation of iron-heart *Cunninghamia lanceolate* germplasm. Experiments have proved that it is feasible and suitable to use the test forest of Chinese fir in Majiang Town as a place

Code	Graft survival rate (%)	Average number of branches	Rootstock trail (cm)	Rootstock height (cm)	Average total growth (cm)
TXS-1	88	7	3.50	32.10	53.40
TXS-2	67	6	2.18	19.00	38.68
TXS-3	70	7	2.76	40.11	41.59
TXS-4	69	8	2.47	19.45	19.83
TXS-5	69	5	1.50	34.00	17.00
TXS-6	85	6	2.77	28.25	24.93
TXS-7	80	7	1.87	16.67	27.67
TXS-8	80	8	1.90	12.30	24.50
TXS-9	90	9	2.35	19.35	38.50
TXS-10	70	5	2.09	20.36	28.00
TXS-11	95	6	2.59	36.88	23.90
TXS-12	85	5	3.23	35.63	33.75
TXS-13	86	6	2.74	33.99	43.91
TXS-14	88	7	2.85	34.61	28.35
TXS-15	89	8	3.51	31.66	35.55
TXS-16	95	7	2.40	32.00	25.30
TXS-17	79	6	2.83	35.00	49.13
TXS-18	89	7	2.40	34.33	34.67
TXS-19	93	8	2.10	28.00	16.00
TXS-20	88	9	2.15	31.00	30.03
TXS-21	87	9	3.00	26.00	49.75
TXS-22	87	7	2.00	20.87	28.82
TXS-23	92	7	2.59	35.59	36.88
TXS-24	91	6	2.77	41.04	42.34
TXS-25	95	7	2.46	21.15	25.35
TXS-26	89	8	3.46	23.66	26.50
TXS-27	89	9	3.03	27.00	35.57
TXS-28	93	7	1.90	20.50	51.30
TXS-29	92	6	2.65	32.35	22.34
TXS-30	96	7	3.10	34.50	31.55
TXS-31	94	8	2.13	20.85	27.15
TXS-32	76	9	2.74	21.70	18.58
TXS-33	77	10	2.56	30.38	24.16
TXS-34	65	8	3.45	41.90	28.55
TXS-35	50	9	1.86	21.00	28.92

Table 8.
Statistics of grafting survival rate of iron-heart Cunninghamia lanceolate.

where the iron-heart *Cunninghamia lanceolate* is preserved in a different place, and the method of splitting can realize the clonal reproduction of iron-heart *Cunninghamia lanceolate* and has a higher survival rate.

3.3 Analysis of sub-populations genetic structure

3.3.1 Genetic diversity

The evolutionary potential and adaptation of a species are reflected by its genetic diversity, the more genetic variation a species has, the more adaptive it is. The study of the genetic diversity of iron-heart *Cunninghamia lanceolate* is necessary to understand its biological characteristics. In total, 133 alleles were observed among all samples for 15 polymorphic loci, which is higher than the amount previously reported. This difference may have been caused by the sample size, reproductive properties, and molecular marker characteristics of the species. The microsatellites used in the study yielded moderately to highly variable allele numbers per locus, in which 15 SSR primer pairs generated a total of 133 alleles, with a mean of 8.87 alleles at each locus, ranging from 5 for the contig5410_1886A locus to 18 for the contig406_1209 locus, except the two loci CLSSR6 and CLSSR8. Both the CLSSR6 locus and CLSSR8 locus had only 2 alleles, producing the lowest Ne (0.641, 0.691). The expected and observed heterozygosity of all the loci ranged from 0.442 to 0.870 and from 0.270 to 0.700, with averages of 0.654 and 0.474, respectively (**Table 9**). As an important index for measuring the genetic diversity of a population, the He of the SSRs was 0.654, which indicated that a higher genetic diversity existed in the population, suggesting that these accessions varied with high genetic diversity. The high genetic diversity may be due to being a predominantly outcrossing species. Meanwhile, the Ne was significantly smaller than the Na for each loci, which may be because the natural ecological conditions became severe suddenly during the process of alternation generation because of the high altitude of the site, and collapse of the large population occurred, leading to the loss of rare alleles in the population and the bottleneck effect. The results also revealed a range of PIC values from 0.348 (CLEER6) to 0.858 (contig406_1209C), and among these, the values of three loci (contig476_526D, 0.421; CLSSR6, 0.348; and CLSSR8, 0.374) were less than 0.5, indicating that the other 12 primers were accessible for identifying the genetic diversity of Chinese fir in Xioxi, Hunan Province. The average Shannon's Information Index (I) value was 1.350, with a minimum of 0.285 (contig 406_1209C) and a maximum of 0.641 (CLSSR8). However, the effective number of alleles (Ne) ranged from 1.792 to 7.677 per locus for all accessions, and the mean value was 3.325. Overall, the mean values of Ne, He, Ho, PIC, Fst, and Gst were 1.933, 0.654, 0.474, 0.566, 0.090, and 0.076, respectively.

There were high levels of differentiation and genetic diversity at these loci. The 15 polymorphic loci showed that the G'stN value was between 0.259 (contig6319_250C) and −0.001 (CLSSR6), with an average value of 0.083. This finding shows that the genetic difference among populations was 8.3%, and 91.7% of the genetic difference existed among individuals in the population. The average Nm of 15 SSR loci in nine populations was 9.163, indicating that gene exchange was frequent.

Na: Number of alleles; Ne: effective number of alleles; I: shannon's Information Index; Ho: Observed heterozygosity; He: Expected heterozygosity with populations; G'stN: Nei's standardized Gst; PIC: The polymorphism information content; Nm = [(1/Fst)-1]/4; Fis (Inbreeding coefficient within individuals) = (Hs-Ho)/Hs; Fst (Inbreeding coefficient within subpopulations) = (Ht-Hs)/Ht; Gis (Analog of Fst, adjusted for bias) = (cHs-Ho)/cHs; Gst (Analog of Fst, adjusted for bias) = (cHt-cHs)/cHt.***

The highest number of alleles was observed in population JZW-3 (Na = 8), and three populations (LYP-2, LYP-3, and LYP-4) had the lowest number of alleles, which was only 4. The observed heterozygosity within a population ranged from

Locus	Na	Ne	I	Ho	He	G'stN	PIC	Nm	Fis	Fit	Fst	Gst
contig476_526D	6	1.792	0.948	0.429	0.442	0.009	0.421	11.812	0.037	0.057	0.021	0.008
contig7616_683B	16	4.088	1.680	0.700	0.755	0.015	0.724	9.161	0.031	0.057	0.027	0.013
contig4728_384B	14	2.304	1.340	0.548	0.566	0.009	0.548	12.039	−0.002	0.018	0.020	0.008
contig5410_1886A	5	3.879	1.389	0.592	0.742	0.042	0.695	4.600	0.193	0.234	0.052	0.038
contig16181_1285C	7	2.811	1.220	0.598	0.644	0.002	0.577	16.098	0.037	0.051	0.015	0.002
contig406_1209C	18	7.677	2.285	0.657	0.870	0.019	0.858	7.211	0.199	0.225	0.034	0.017
Unigene633	7	3.878	1.488	0.391	0.742	0.206	0.701	0.997	0.304	0.444	0.200	0.187
Unigene754	5	3.148	1.282	0.422	0.682	0.232	0.626	0.866	0.201	0.380	0.224	0.212
Unigene840	7	3.081	1.340	0.644	0.675	0.075	0.630	2.948	−0.027	0.053	0.078	0.067
Unigene1061	10	4.504	1.691	0.369	0.778	0.106	0.746	1.961	0.514	0.569	0.113	0.095
Unigene491	12	3.208	1.591	0.270	0.688	0.160	0.652	1.298	0.527	0.603	0.162	0.145
contig6319_250C	7	2.335	1.105	0.448	0.572	0.259	0.524	0.751	−0.082	0.189	0.250	0.237
contig1560_1789D	15	3.368	1.551	0.423	0.703	0.131	0.661	1.614	0.397	0.478	0.134	0.119
CLSSR6	2	1.815	0.641	0.332	0.449	−0.001	0.348	17.026	0.238	0.249	0.014	−0.001
CLSSR8	2	1.993	0.691	0.290	0.498	−0.014	0.374	49.057	0.413	0.416	0.005	−0.012
Mean	8.87	3.325	1.350	0.474	0.654	0.083	0.566	9.163	0.199	0.268	0.090	0.076

Table 9.
Characterization of 15 simple sequence repeat loci in iron-heart Cunninghamia lanceolate *based on 548 accessions representing 9 sampling sites.*

0.416 to 0.506, varying little. The mean of the expected heterozygosity within populations was significantly higher than the observed heterozygosity (Ho) within populations, while the highest value was found for population LYP-1 (He = 0.637), and the lowest value of 0.524 was found in LYP-4 (**Table S2**). LYP-4 was the least diverse population (I = 0.997 and He = 0.524) of all the sites sampled. The highest genetic diversity was recorded for sites located in JWZ-2, JWZ-3, and LYP-1 (I = 1.244, 1.294, and 1.241 and He = 0.622, 0.636, and 0.637, respectively). In **Figure 3**, the geographic distribution of the population diversity based on Fp and He is presented, which indicated that JZW- (1,2,3) was likely the center of genetic diversity of this Chinese fir variety.

Molecular variance analysis was used to assess the population differentiation among 9 subgroups, which demonstrated that approximately 11% of the total variance was explained among the groups and 89% of the total variance was explained within accessions (**Table 10**). The population differentiation study that included red-heartwood Chinese fir and clones from six different provinces produced similar results to our study and identified a slightly higher genetic variance in subgroups. However, a moderate degree of variability was present among some populations. Previous studies [38] have shown that severe genetic drift, which might be intensified by long-term habitat isolation, is widespread in small populations. This effect will result in a low level of genetic diversity within a population and genetic differentiation among populations. Meanwhile, the results were almost consistent with G'stN = 0.083, indicating that variation mainly existed between individuals, so it was unreasonable to divide the groups according to geographical locations and administrative boundaries.

3.3.2 Genetic structure and divergence

The study of population structure is important for the formulation of strategies utilizing special germplasms for breeding objectives and conserving species effectively. Meanwhile, the genetic structure largely determines the evolutionary potential of a species or population. To verify the results of the neighbor-joining cluster analysis and PCA principal component analysis, the results of 15 pairs of SSR primer polymorphisms of 548 wild germplasm resources in Xiaoxi, Hunan Province, were further analyzed by STRUCTURE v2.3.4. The results showed that L(K) increased with the increase of K. A clear peak appeared at the value of ΔK at K = 2 (**Figure 4A** and **B**). When k = 2, ΔK reached the peak value, which indicated that the 548 accessions were clearly differentiated into two clusters according to STRUCTURE analysis (**Figure 4**). All the accessions from JZW-2, JZW-3, and LYP-1 were present

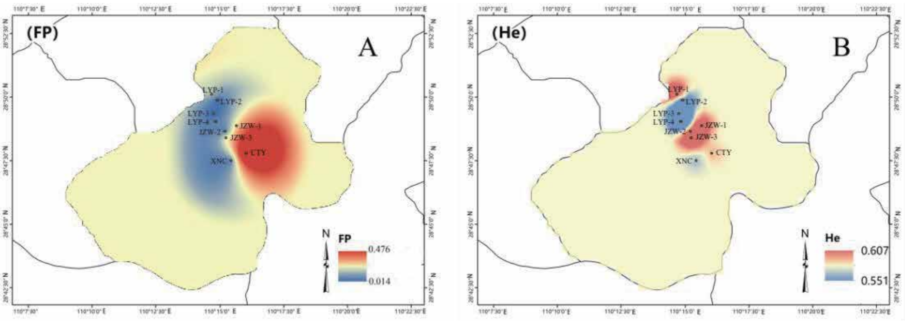

Figure 3.
Distribution of population diversity based on the expected heterozygosity and private allele frequency. (A) The private allele frequency (Fp) in all populations. (B) The expected heterozygosity (He) in all populations.

Source of variation	Degree of freedom	Sum of squares	Variance component estimates	Percentage of variation (%)
Among populations	9	804.900	1.541	11
Between samples within populations	539	6443.894	11.955	89
Total	548	7248.794	13.496	100

Table 10.
Analysis of molecular variance (AMOVA) among populations of iron-heart Cunninghamia lanceolate.

in two clusters, with approximately one-half of each population in each cluster, which can be considered admixed. Materials from different sources were distributed in the populations, there was no obvious regional differentiation, and the results of the population structure analysis were consistent with the results of SSR genetic diversity clustering. According to previous research, in the genetic structure analysis of a structured population, when the genetic component (Q value) of material is ≥0.6, the genetic background of the material is relatively simple, and when the Q value is <0.6, the genetic background of the material is relatively complex. With the increase of the K value (k = 3, k = 4), a new gene classification appeared in the wild Chinese fir population, but the high variance was inconsistent (**Figure 4D**). The clustering of CTY, JZW-1, JZW-2, JZW-3, and XNC showed some evidence that these populations can be broken down into further clusters, while LYP-1, LYP-2, LYP-3, and LYP-4 were relatively stable for higher K values. Excluding the CTY and JZW-1 populations, a new gene classification appeared in the other seven populations, which showed that there were significant differences among other populations. This finding suggested that the heterozygosity and genetic background of the wild Chinese fir are higher. When K = 4, the population was divided into four groups. The accessions that originated from the same population, including JZW-1, JZW-3, and XNC, were divided into different clusters. This result indicated that the four clusters are not geographically independent. Several populations (i.e., the LYP-3 and LYP-4 populations) that consisted of a single genetic component might have experienced founder effects or significant bottlenecking. The results also show low levels of mixing, which account for the hybridization or outcrossing of individuals between populations. Classifying accessions according to administrative boundaries and geographical distributions is very subjective, and it is very difficult to grade traits accurately in the provenance of this specific Chinese fir. In some cases, the population structure may not be predicted via administrative boundaries and geographical distributions. Therefore, the relationship between the population structure and phylogenetic clustering is not obvious, which is consistent with previous research results [22] for the Chinese fir. Wind pollination and a high natural outcrossing frequency among the species may lead to inconsistencies in population classifications and geographical locations. As a result, the geographical origin and genetic structure of a population should be simultaneously considered for the screening of this special germplasm breeding material. That is, geographical features are not obvious among distribution regions. From the principal component analysis results, we were able to identify two main populations with some sub-populations in each group. Obviously, the distributions of accessions from the same location in the two groups were not concentrated and scattered in each group. Additionally, one group contained all the individuals from JZW-1 and approximately 60% of the accessions from the other three locations (ZJW-2, JZW-3, and LYP-1), which occupied approximately 40% in the other group.

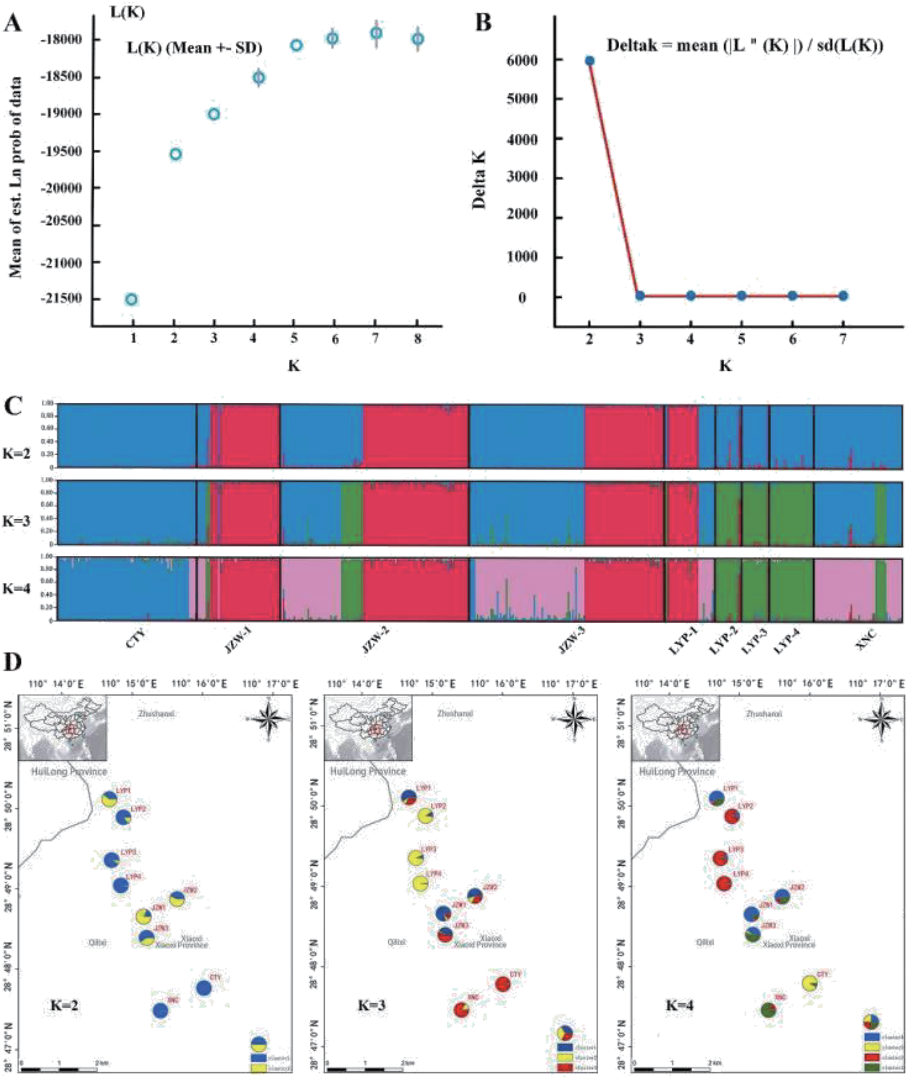

Figure 4.

Population classification based on the consensus of STRUCTURE analysis across 10 replications for per K clusters. (A) Circles with standard deviations represent the average log-likelihoods across per K runs independently. (B) Solid circles indicate the values of Evanno's ΔK based on the rate of change of the log-likelihood. (C) Bar plots express the population structure. The number of clusters is shown from K = 2 to K = 4. Vertical bars represent each genotype, and the length of each colored bar represents the proportion of membership for each cluster. (D) the distribution of 2 to 4 clusters of 9 populations is visualized as a pie chart, with each population divided into colored segments based on the proportion of its members in a given cluster.

The lowest Gst and Fst values between populations JWZ-2 and LYP1 were 0.004 and 0.010, respectively (**Table S3**). The highest values, which were 0.104 for Fst and 0.093 for Gst, were observed between populations CTY and LYP-2. Most of the values for both parameters were within the limits of moderate genetic differentiation between populations (**Table S3**).

4. Discussion

Seed yield and quality are the basis for the collection and preservation of improved seeds and the construction of seed orchards, which has a great impact on the efficiency of plantation and industrial development in the later stage [39, 40]. It is found that the variation range of seed and cone traits is 12.18–51.34%, among which the variation of cone length, cone width, cone length-width ratio, seed length, seed width, and seed length-width ratio is relatively small, indicating that these seed traits of iron-heart *Cunninghamia lanceolate* are relatively stable [41, 42]. The order of coefficient of variation from large to small is: seed germination rate > seed quality > seed length to width ratio > seed length > cone seed yield > seed width > total cone mass > seed goodness > cone length > seed quality (1000) > cone length to width ratio > cone width. The results of the analysis of variance showed that among families, the differences of total cone quality, seed quality, seed germination rate, seed goodness, seed quality (1000), seed width, and cone length-width ratio were very significant ($P < 0.001$), the differences of cone length were significant ($P < 0.01$), and the differences of the other four traits were not significant. The results showed that the phenotypic characters of different Iron-heart *Cunninghamia lanceolate* families had high diversity and rich variation.

Genetic diversity of a species reflects its evolutionary potential and allows for evolution and adaptation. The more abundant the genetic variation of a species is, the more adaptable it is. Thus, it is necessary to study the genetic diversity of a species to understand its biological properties [43]. All previous studies on this species revealed a relatively high level of genetic diversity [22]. In the current study, 15 SSR markers were used to evaluate the population genetics of a large number of specific Chinese fir individuals across its distribution range in Xiaoxi Hunan. Amplification results of the 548 germplasms only existed Hunan Xiaoxi gave a total of 133 alleles with a mean of 8.87 at each locus, a value higher than those in previous reports [1, 22]. The difference may relate to the reproductive attributes of this species, the sample size, and/or the characteristics of the molecular markers. Understanding population structure is useful for developing strategies for the conservation of new species and effectively utilizing genotypes for breeding purposes. Genetic distance is commonly used to describe the genetic structure of a population and the differences among populations [44]. The evolutionary potential of a species or population depends to a large extent on the genetic structure of the population [45]. The results of the STRUCTURE analysis performed for this study indicate that the most likely genetic structure of the 548 studied accessions is two clusters.

5. Conclusions

Through this study, we constructed a germplasm resource nursery of Iron-heart China fir, and the grafting survival rate was as high as 83%. 27 families of iron-heart *Cunninghamia lanceolate* seeds were collected, and the highest germination rate was 68%; 15 highly polymorphic and stable SSR markers were selected to analyze the genetic structure of the natural population of iron-heart *Cunninghamia lanceolate*. In total, the study got 133 alleles, and the GestN's = 0.083. AMOVA analysis showed that the variation among populations was only 11%, and 89% of the variation came from individuals. In addition, STRUCTURE analysis showed that the whole samples could be divided into two groups, and there was no correlation between population division and geographical location. This study will lay a foundation for the protection of the new species of Iron-heart *Cunninghamia lanceolate*. In this study, only the genetic structure of its natural population was analyzed, but the heartwood variation was not deeply discussed. In addition, we only used the single method of STRUCTURE to analyze its genetic structure and did not use PCA, neighbor-joining (NJ) cluster analysis, and other methods to analyze its genetic structure. This will be what we will study in the next step.

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A. Summary materials

Gst values above the diagonal; Fst values below the diagonal.

Code	DBH (cm)	Altitude (m)	GPS(E,N)
TXS-1	72.00	895	110.246958, 28.835133
TXS-2	48.20	899	110.247068, 28.835290
TXS-3	41.10	845	110.247144, 28.833239
TXS-4	43.20	505	110.260574, 28.814858
TXS-5	29.50	560	110.260804, 28.814984
TXS-6	44.10	563	110.260804, 28.814984
TXS-7	63.00	813	110.246442, 28.822674
TXS-8	25.30	804	110.246186, 28.822969
TXS-9	51.50	800	110.247032, 28.823265
TXS-10	47.00	807	110.247682, 28.823303
TXS-11	43.00	894	110.245943, 28.834792
TXS-12	35.50	1000	110.242849, 28.834510
TXS-13	37.40	1010	110.243119, 28.835489
TXS-14	47.00	904	110.245980, 28.835239
TXS-15	46.00	894	110.246676, 28.834436
TXS-16	43.20	648	110.268425, 28.798110
TXS-17	26.90	632	110.268312, 28.797980
TXS-18	27.70	648	110.268386, 28.798233
TXS-19	40.60	632	110.267831, 28.797993
TXS-20	34.40	629	110.267767, 28.796707
TXS-21	54.70	902	110.247119, 28.835550
TXS-22	54.20	901	110.246885, 28.835296
TXS-23	73.00	895	110.246958, 28.835133
TXS-24	48.20	899	110.247968, 28.835290
TXS-25	41.10	845	110.247444, 28.833239
TXS-26	43.20	505	110.260574, 28.814858
TXS-27	29.50	560	110.260804, 28.814984
TXS-28	52.30	490	110.261149, 28.814843
TXS-29	38.70	514	110.261320, 28.814457
TXS-30	27.30	511	110.261126, 28.814625

Code	DBH (cm)	Altitude (m)	GPS(E,N)
TXS-31	29.40	524	110.260469, 28.814065
TXS-32	29.00	502	110.259730, 28.813559
TXS-33	37.70	494	110.269097, 28.814165
TXS-34	28.00	525	110.259179, 28.814850
TXS-35	31.80	523	110.250914, 28.814820

Table S1.
The information of the 35 Iron-heart Cunninghamia lanceolate.

Code	Plot	Position	longitude	latitude	Altitude(m)
1	1	CTY	110.266812	28.793035	534
2	1	CTY	110.266115	28.794041	541
3	1	CTY	110.266582	28.795033	544
4	1	CTY	110.267011	28.794845	551
5	1	CTY	110.267154	28.795096	514
6	1	CTY	110.267154	28.795096	561
7	1	CTY	110.2683	28.794092	605
8	1	CTY	110.2683	28.794092	596
9	1	CTY	110.269446	28.794093	602
10	1	CTY	110.269446	28.794093	616
14	1	CTY	110.271739	28.793089	654
15	4	JZW3	110.263718	28.803132	658
16	4	JZW3	110.264863	28.804133	655
17	1	CTY	110.269446	28.792084	660
18	4	JZW3	110.264863	28.804133	653
19	1	CTY	110.274031	28.792087	652
20	1	CTY	110.274031	28.792087	654
21	1	CTY	110.274031	28.792087	656
22	1	CTY	110.274031	28.792087	661
23	4	JZW3	110.262572	28.804132	660
24	1	CTY	110.271739	28.794094	659
25	1	CTY	110.271739	28.794094	644
26	1	CTY	110.272311	28.793592	646
27	1	CTY	110.272549	28.79341	664
28	1	CTY	110.272455	28.793361	650
29	1	CTY	110.272322	28.793322	651
30	1	CTY	110.272446	28.793236	645
31	1	CTY	110.272293	28.793381	642
32	1	CTY	110.272159	28.793616	623
33	1	CTY	110.272141	28.793757	653

Code	Plot	Position	longitude	latitude	Altitude(m)
34	1	CTY	110.27215	28.793773	654
35	1	CTY	110.272276	28.793624	663
36	1	CTY	110.272132	28.79378	658
37	1	CTY	110.272061	28.793863	668
38	1	CTY	110.272061	28.793863	663
39	1	CTY	110.272178	28.793881	655
40	1	CTY	110.272162	28.793761	661
41	1	CTY	110.272151	28.796853	666
42	1	CTY	110.272276	28.794812	657
43	1	CTY	110.269445	28.788067	650
44	1	CTY	110.272123	28.794133	657
45	1	CTY	110.272101	28.793965	645
46	1	CTY	110.27215	28.794034	646
47	1	CTY	110.272509	28.794644	630
48	1	CTY	110.272473	28.794675	627
49	1	CTY	110.272419	28.794675	625
50	1	CTY	110.272401	28.794754	630
51	1	CTY	110.272491	28.794675	632
52	1	CTY	110.272347	28.794769	635
53	1	CTY	110.272419	28.794691	645
54	1	CTY	110.272437	28.794738	645
55	1	CTY	110.272419	28.794691	650
56	1	CTY	110.272456	28.794738	651
57	1	CTY	110.272456	28.794973	650
58	1	CTY	110.272599	28.79491	653
59	1	CTY	110.272813	28.794754	653
60	1	CTY	110.272706	28.794722	638
61	1	CTY	110.272675	28.794859	675
62	1	CTY	110.272804	28.794785	655
63	1	CTY	110.272666	28.794701	673
64	1	CTY	110.27274	28.794741	675
65	1	CTY	110.273003	28.794652	676
66	1	CTY	110.272473	28.794785	677
67	1	CTY	110.273064	28.794565	711
68	1	CTY	110.273064	28.794565	680
69	1	CTY	110.273064	28.794565	688
70	1	CTY	110.273261	28.794511	681
71	1	CTY	110.273097	28.794544	674
72	1	CTY	110.273178	28.794603	668
73	1	CTY	110.273231	28.794833	655

Code	Plot	Position	longitude	latitude	Altitude(m)
74	1	CTY	110.273231	28.948323	671
75	1	CTY	110.273214	28.794826	668
76	1	CTY	110.273208	28.794858	658
77	1	CTY	110.27334	28.794583	667
78	1	CTY	110.27344	28.794662	663
79	1	CTY	110.273415	28.794634	662
80	1	CTY	110.273119	28.794773	660
81	1	CTY	110.273501	28.794945	679
82	1	CTY	110.273369	28.794766	675
83	1	CTY	110.273626	28.795024	613
84	1	CTY	110.27365	28.795094	630
85	1	CTY	110.273573	28.795127	657
86	1	CTY	110.273598	28.795052	665
87	1	CTY	110.273357	28.79507	653
88	1	CTY	110.273261	28.795105	789
89	1	CTY	110.27327	28.794935	870
90	1	CTY	110.273327	28.794876	759
91	1	CTY	110.273147	28.795255	667
92	1	CTY	110.272935	28.795291	682
93	1	CTY	110.273253	28.795221	660
94	1	CTY	110.27328	28.795224	674
95	1	CTY	110.273167	28.795279	655
97	1	CTY	110.27311	28.795333	666
98	1	CTY	110.273139	28.79538	691
99	1	CTY	110.273337	28.795049	653
100	1	CTY	110.273321	28.794943	635
101	2	JZW-1	110.258688	28.811154	486
102	2	JZW-1	110.25895	28.811245	482
103	3	JZW-2	110.261468	28.812119	496
104	3	JZW-2	110.261475	28.812175	504
107	3	JZW-2	110.261663	28.81301	549
108	3	JZW-2	110.261508	28.813028	526
109	3	JZW-2	110.26122	28.813561	527
110	3	JZW-2	110.261226	28.813602	520
112	3	JZW-2	110.260904	28.81422	509
113	3	JZW-2	110.260998	28.814105	506
114	3	JZW-2	110.260803	28.814323	497
115	3	JZW-2	110.260723	28.814317	482
117	3	JZW-2	110.26169	28.815117	507
118	3	JZW-2	110.262119	28.815117	500

Code	Plot	Position	longitude	latitude	Altitude(m)
119	3	JZW-2	110.26169	28.815493	510
120	3	JZW-2	110.26169	28.815493	509
121	3	JZW-2	110.26169	28.815493	515
122	3	JZW-2	110.26169	28.81474	526
123	3	JZW-2	110.262549	28.81474	539
124	3	JZW-2	110.262549	28.81474	513
125	3	JZW-2	110.262549	28.81474	519
127	3	JZW-2	110.263409	28.81474	531
128	3	JZW-2	110.26169	28.81474	534
129	3	JZW-2	110.26169	28.81474	535
130	3	JZW-2	110.263409	28.81474	537
131	3	JZW-2	110.263409	28.81474	531
132	3	JZW-2	110.263409	28.81474	552
134	3	JZW-2	110.26169	28.81474	538
136	3	JZW-2	110.26169	28.81474	525
137	3	JZW-2	110.26169	28.81474	521
138	3	JZW-2	110.26169	28.81474	537
139	3	JZW-2	110.26169	28.81474	535
140	3	JZW2	110.26169	28.81474	528
141	3	JZW-2	110.26169	28.81474	519
142	3	JZW-2	110.26169	28.81474	524
143	3	JZW-2	110.26169	28.81474	523
144	2	JZW-1	110.256532	28.813234	522
145	3	JZW-2	110.26169	28.81474	514
146	3	JZW-2	110.26169	28.81474	525
147	3	JZW2	110.26169	28.81474	513
148	3	JZW-2	110.26169	28.81474	510
149	3	JZW-2	110.26169	28.81474	511
150	3	JZW-2	110.26169	28.81474	512
151	9	XNC	110.25808	28.794866	426
152	9	XNC	110.258057	28.794869	425
153	9	XNC	110.25805	28.794869	427
154	9	XNC	110.257734	28.792924	352
155	2	JZW-1	110.255928	28.81311	500
156	2	JZW-1	110.255961	28.812925	513
157	9	XNC	110.257942	28.792232	496
158	9	XNC	110.257963	28.792241	499
159	9	XNC	110.258036	28.792211	495
160	9	XNC	110.257983	28.792156	492
161	9	XNC	110.256324	28.792974	556

Code	Plot	Position	longitude	latitude	Altitude(m)
162	9	XNC	110.25626	28.793065	554
163	9	XNC	110.256173	28.793191	519
164	9	XNC	110.256173	28.793197	547
165	9	XNC	110.25619	28.793335	548
166	9	XNC	110.256159	28.7934	545
167	9	XNC	110.256149	28.793409	548
168	9	XNC	110.256129	28.793385	548
169	9	XNC	110.256116	28.793521	531
170	9	XNC	110.256096	28.793444	543
171	9	XNC	110.256096	28.793556	542
172	9	XNC	110.256069	28.793668	533
173	9	XNC	110.256089	28.793644	534
174	9	XNC	110.256109	28.793574	530
175	9	XNC	110.256123	28.793633	527
176	9	XNC	110.256129	28.793627	523
177	9	XNC	110.256096	28.793739	520
178	9	XNC	110.256082	28.793733	515
179	9	XNC	110.255975	28.793344	500
180	9	XNC	110.256076	28.79355	526
181	9	XNC	110.25668	28.791991	534
182	9	XNC	110.256928	28.792044	542
183	9	XNC	110.256814	28.791926	535
184	9	XNC	110.256754	28.791779	527
185	9	XNC	110.2567	28.791744	524
186	9	XNC	110.256673	28.791732	538
187	9	XNC	110.256512	28.791849	526
188	9	XNC	110.256458	28.791808	526
189	9	XNC	110.256458	28.79182	532
190	9	XNC	110.256519	28.791908	530
191	9	XNC	110.256465	28.791902	540
192	9	XNC	110.256378	28.791745	536
194	9	XNC	110.256438	28.791685	542
195	9	XNC	110.256371	28.791633	556
196	9	XNC	110.256358	28.791508	553
197	9	XNC	110.256445	28.791402	552
198	9	XNC	110.256438	28.79139	552
199	9	XNC	110.255995	28.791137	544
200	9	XNC	110.25621	28.790978	545
201	5	LYP-1	110.251672	28.831943	928
202	5	LYP-1	110.251716	28.83204	920

Code	Plot	Position	longitude	latitude	Altitude(m)
203	6	LYP-2	110.251766	28.830129	862
204	6	LYP-2	110.251838	28.830039	882
205	6	LYP-2	110.251952	28.829865	845
206	6	LYP-2	110.251776	28.829784	872
207	6	LYP-2	110.251688	28.829844	871
208	6	LYP-2	110.252861	28.829196	796
210	6	LYP-2	110.253689	28.828283	755
211	6	LYP-2	110.253985	28.827786	749
212	6	LYP-2	110.253501	28.827892	730
213	6	LYP-2	110.254058	28.827841	738
214	6	LYP-2	110.253728	28.827494	733
215	6	LYP-2	110.254178	28.827329	733
216	6	LYP-2	110.254169	28.827263	734
217	6	LYP-2	110.254318	28.827022	728
218	6	LYP-2	110.254701	28.826678	710
220	7	LYP-3	110.251102	28.819344	879
221	7	LYP-3	110.250925	28.819311	889
222	7	LYP-3	110.251247	28.819485	860
223	7	LYP-3	110.25086	28.819912	875
224	7	LYP-3	110.250989	28.820227	878
225	7	LYP-3	110.250962	28.819546	870
226	7	LYP-3	110.251204	28.819504	840
228	7	LYP-3	110.25115	28.819278	835
229	7	LYP-3	110.251005	28.81924	837
230	7	LYP-3	110.251354	28.819156	830
232	7	LYP-3	110.251451	28.819231	830
234	7	LYP-3	110.251268	28.818977	814
236	7	LYP-3	110.2513	28.818902	733
237	7	LYP-3	110.251434	28.818902	678
238	7	LYP-3	110.251366	28.818901	810
239	7	LYP-3	110.251472	28.818635	807
240	7	LYP-3	110.251367	28.818623	806
241	8	LYP-4	110.253403	28.814614	785
242	8	LYP-4	110.253464	28.814627	780
243	8	LYP-4	110.253504	28.814843	780
244	8	LYP-4	110.253303	28.814612	790
245	8	LYP-4	110.252381	28.814914	787
246	8	LYP4	110.252431	28.814457	787
247	8	LYP-4	110.252565	28.814573	785
248	8	LYP-4	110.252531	28.814643	770

Code	Plot	Position	longitude	latitude	Altitude(m)
249	8	LYP-4	110.252415	28.814638	780
250	8	LYP-4	110.25249	28.81451	790
251	8	LYP-4	110.252389	28.814524	780
252	8	LYP-4	110.252617	28.814707	780
253	8	LYP-4	110.252464	28.814759	822
254	8	LYP-4	110.252586	28.814907	823
255	8	LYP-4	110.252485	28.814505	815
256	8	LYP-4	110.252474	28.81493	825
257	8	LYP-4	110.252442	28.81495	826
258	8	LYP-4	110.252474	28.81493	825
259	8	LYP-4	110.252432	28.81475	836
260	2	JZW-1	110.259991	28.808601	830
261	2	JZW-1	110.259993	28.808731	819
263	8	LYP-4	110.251947	28.814613	824
264	8	LYP-4	110.251909	28.814514	837
265	8	LYP-4	110.251896	28.814477	840
266	8	LYP-4	110.251815	28.814618	834
267	8	LYP-4	110.25207	28.81447	825
268	8	LYP-4	110.252282	28.814319	819
269	8	LYP-4	110.252118	28.814293	821
270	8	LYP-4	110.252175	28.814322	821
271	8	LYP-4	110.252244	28.814284	815
272	8	LYP-4	110.251362	28.814845	799
273	8	LYP-4	110.251322	28.814858	808
274	8	LYP-4	110.251597	28.813805	808
275	8	LYP-4	110.2515	28.813742	823
276	3	JZW-2	110.264737	28.810652	525
277	3	JZW-2	110.260078	28.814223	485
278	3	JZW-2	110.260078	28.814175	488
279	3	JZW-2	110.260024	28.814317	491
280	3	JZW-2	110.25997	28.814364	509
281	3	JZW-2	110.259863	28.814599	504
282	3	JZW-2	110.259863	28.814599	504
283	3	JZW-2	110.259809	28.814646	510
284	3	JZW-2	110.259809	28.814693	514
285	3	JZW-2	110.259702	28.814787	513
286	3	JZW-2	110.259756	28.814787	513
287	3	JZW-2	110.259702	28.814787	518
288	3	JZW-2	110.259648	28.814787	520
289	3	JZW-2	110.259648	28.814882	525

Code	Plot	Position	longitude	latitude	Altitude(m)
291	3	JZW-2	110.259326	28.814976	535
293	3	JZW-2	110.259218	28.814882	525
295	3	JZW-2	110.259218	28.814699	523
296	3	JZW-2	110.259218	28.814599	526
297	3	JZW-2	110.259326	28.814552	516
298	3	JZW-2	110.259433	28.814458	510
299	3	JZW-2	110.259863	28.813611	464
300	2	JZW-1	110.263011	28.80788	498
301	2	JZW-1	110.263	28.808087	503
302	2	JZW-1	110.263183	28.808167	506
303	2	JZW-1	110.263571	28.808077	510
304	3	JZW-2	110.265989	28.811247	545
305	3	JZW-2	110.266004	28.811278	576
306	3	JZW-2	110.266103	28.811241	523
307	3	JZW-2	110.266153	28.811309	535
308	3	JZW-2	110.266199	28.811258	537
309	3	JZW-2	110.266199	28.811259	537
310	3	JZW-2	110.266079	28.811436	520
311	3	JZW-2	110.266025	28.811335	520
312	3	JZW-2	110.265996	28.811373	532
313	3	JZW-2	110.265996	28.811372	520
314	3	JZW-2	110.265858	28.811433	528
315	3	JZW-2	110.265956	28.81142	531
316	3	JZW-2	110.265863	28.811323	529
317	3	JZW-2	110.265504	28.811433	525
318	3	JZW-2	110.265889	28.81137	529
319	3	JZW-2	110.265938	28.811365	530
320	3	JZW-2	110.265968	28.81137	531
321	3	JZW-2	110.265889	28.811301	529
323	3	JZW-2	110.265745	28.81161	526
324	3	JZW-2	110.265873	28.811646	525
325	3	JZW-2	110.265485	28.811865	536
326	3	JZW-2	110.265524	28.811862	537
327	3	JZW-2	110.265556	28.811825	537
328	3	JZW-2	110.265618	28.811889	526
330	3	JZW-2	110.265554	28.811823	530
331	3	JZW-2	110.265658	28.811802	530
332	3	JZW-2	110.265503	28.811767	535
333	3	JZW-2	110.265296	28.811861	557
334	3	JZW-2	110.265234	28.811896	539

Code	Plot	Position	longitude	latitude	Altitude(m)
335	7	LYP-3	110.265138	28.811964	538
335	3	JZW-2	110.265138	28.811964	538
336	3	JZW-2	110.264905	28.811888	542
337	3	JZW-2	110.264886	28.811812	535
338	3	JZW-2	110.264902	28.811727	537
339	3	JZW-2	110.264918	28.811686	543
340	3	JZW-2	110.265194	28.811692	538
341	3	JZW-2	110.265055	28.811593	540
342	3	JZW-2	110.265127	28.811549	532
343	3	JZW-2	110.265103	28.811499	532
344	3	JZW-2	110.265036	28.811453	535
345	3	JZW-2	110.265021	28.811431	540
346	3	JZW-2	110.265059	28.811403	538
347	3	JZW-2	110.264996	28.811403	535
348	3	JZW-2	110.265917	28.811525	542
349	3	JZW-2	110.265937	28.811543	525
350	3	JZW-2	110.265909	28.811615	529
351	3	JZW-2	110.266003	28.811554	530
352	3	JZW-2	110.266267	28.811835	540
353	3	JZW-2	110.266255	28.811816	533
354	3	JZW-2	110.266272	28.811721	541
355	3	JZW-2	110.266164	28.811771	538
356	3	JZW-2	110.265991	28.811558	532
357	3	JZW-2	110.266228	28.811586	540
358	3	JZW-2	110.266193	28.811927	535
359	3	JZW-2	110.265963	28.811565	545
360	3	JZW-2	110.26604	28.811553	533
362	3	JZW-2	110.266012	28.811557	537
363	6	LYP-2	110.252271	28.829358	818
364	6	LYP-2	110.252219	28.829951	854
365	5	LYP-1	110.251349	28.831102	899
366	5	LYP-1	110.251304	28.831128	899
367	5	LYP-1	110.250948	28.831558	922
368	5	LYP-1	110.250874	28.831577	930
369	5	LYP-1	110.250689	28.831557	932
370	5	LYP-1	110.250634	28.832051	900
371	5	LYP-1	110.250478	28.832025	940
372	5	LYP-1	110.250434	28.832028	940
373	2	JZW-1	110.263912	28.808815	945
374	5	LYP-1	110.250163	28.832358	945

Code	Plot	Position	longitude	latitude	Altitude(m)
375	5	LYP-1	110.249569	28.832626	998
376	5	LYP-1	110.249466	28.832862	1015
377	5	LYP-1	110.249088	28.832724	1030
378	5	LYP-1	110.249297	28.832514	1011
379	5	LYP-1	110.249066	28.832626	1033
380	5	LYP-1	110.248575	28.83256	1035
381	5	LYP-1	110.248598	28.832476	1040
382	5	LYP-1	110.24879	28.832071	1050
383	5	LYP-1	110.248354	28.832044	1028
385	5	LYP-1	110.248287	28.83145	1022
386	5	LYP-1	110.248318	28.831274	1005
389	5	LYP-1	110.248173	28.831164	990
390	5	LYP-1	110.248311	28.831247	991
392	5	LYP-1	110.248629	28.830985	980
393	5	LYP-1	110.248575	28.831086	967
394	5	LYP-1	110.248873	28.831389	950
395	5	LYP-1	110.249581	28.830962	934
396	5	LYP-1	110.249954	28.831276	938
397	5	LYP-1	110.250058	28.831746	937
398	5	LYP-1	110.251929	28.832356	921
399	5	LYP-1	110.251779	28.832887	921
400	5	LYP-1	110.251693	28.832149	921
401	9	XNC	110.256284	28.79092	542
402	9	XNC	110.256519	28.79092	542
403	9	XNC	110.256485	28.790973	540
404	9	XNC	110.256586	28.791055	539
405	9	XNC	110.256586	28.791043	539
406	9	XNC	110.256693	28.790949	538
407	9	XNC	110.256687	28.790949	546
408	9	XNC	110.256942	28.790949	540
409	9	XNC	110.25721	28.790172	513
410	9	XNC	110.257351	28.790431	492
411	4	JZW-3	110.257822	28.804113	467
412	4	JZW-3	110.257472	28.804066	477
413	4	JZW-3	110.257472	28.804066	477
414	4	JZW-3	110.257284	28.803972	491
415	4	JZW-3	110.257123	28.803996	496
416	4	JZW-3	110.257043	28.804031	498
417	4	JZW-3	110.256922	28.804561	524
418	4	JZW-3	110.256855	28.804572	508

Code	Plot	Position	longitude	latitude	Altitude(m)
419	4	JZW-3	110.256761	28.804631	517
420	4	JZW-3	110.25668	28.804678	494
421	4	JZW-3	110.256667	28.804702	515
422	4	JZW-3	110.256613	28.804725	519
423	4	JZW-3	110.256519	28.804737	520
424	4	JZW-3	110.256411	28.804808	512
425	4	JZW-3	110.256304	28.804878	526
426	4	JZW-3	110.25625	28.804867	520
427	4	JZW-3	110.256264	28.80486	526
428	4	JZW-3	110.256129	28.80489	528
429	4	JZW-3	110.256035	28.80489	527
430	4	JZW-3	110.255982	28.804937	530
431	4	JZW-3	110.255901	28.805067	541
432	4	JZW-3	110.25582	28.805055	536
433	4	JZW-3	110.25578	28.805043	537
434	4	JZW-3	110.255753	28.805079	542
435	4	JZW-3	110.255673	28.805149	537
436	4	JZW-3	110.255646	28.805161	538
437	4	JZW-3	110.255485	28.805196	543
438	4	JZW-3	110.255471	28.805208	550
439	4	JZW-3	110.255216	28.805302	558
440	4	JZW-3	110.254894	28.805396	559
441	4	JZW-3	110.254692	28.80542	553
442	4	JZW-3	110.254692	28.805408	565
443	4	JZW-3	110.254571	28.805396	564
444	4	JZW-3	110.25445	28.805396	568
445	4	JZW-3	110.254316	28.805361	566
446	4	JZW-3	110.254236	28.805349	567
447	4	JZW-3	110.254155	28.805337	569
448	4	JZW-3	110.254061	28.80533	570
449	4	JZW-3	110.253954	28.805349	572
450	4	JZW-3	110.253859	28.805361	576
452	4	JZW-3	110.253094	28.805585	579
453	4	JZW-3	110.253121	28.805561	599
454	4	JZW-3	110.253013	28.805632	595
455	4	JZW-3	110.252664	28.805679	611
456	4	JZW-3	110.252557	28.805655	612
457	4	JZW-3	110.252369	28.805702	618
458	4	JZW-3	110.252315	28.805702	622
459	4	JZW-3	110.252208	28.805749	626

Code	Plot	Position	longitude	latitude	Altitude(m)
460	4	JZW-3	110.251885	28.805843	637
461	4	JZW-3	110.251858	28.805867	642
462	4	JZW-3	110.251724	28.805867	649
463	4	JZW-3	110.25167	28.805867	652
464	4	JZW-3	110.251563	28.805941	659
465	4	JZW-3	110.251536	28.805914	654
466	4	JZW-3	110.251429	28.805891	651
467	4	JZW-3	110.251429	28.805914	650
468	4	JZW-3	110.251402	28.805914	648
469	4	JZW-3	110.251348	28.805961	644
470	4	JZW-3	110.251187	28.806008	648
471	4	JZW-3	110.25116	28.806126	652
472	4	JZW-3	110.25116	28.806126	648
473	4	JZW-3	110.25116	28.806102	644
474	4	JZW-3	110.251026	28.807115	641
475	4	JZW-3	110.250918	28.807044	660
476	4	JZW-3	110.250864	28.807185	662
477	4	JZW-3	110.250891	28.807303	658
478	4	JZW-3	110.250891	28.807303	657
479	4	JZW-3	110.250918	28.807303	656
480	4	JZW-3	110.250891	28.80735	654
481	4	JZW-3	110.250811	28.80742	658
482	4	JZW-3	110.250838	28.807444	659
483	2	JZW-1	110.250945	28.81041	671
485	2	JZW-1	110.251052	28.810457	663
486	2	JZW-1	110.251106	28.81048	662
487	2	JZW-1	110.25116	28.810504	663
488	2	JZW-1	110.251267	28.810551	659
489	2	JZW-1	110.251321	28.810551	661
490	2	JZW-1	110.252557	28.810716	629
491	2	JZW-1	110.252637	28.810833	624
492	2	JZW-1	110.252745	28.810904	624
493	2	JZW-1	110.253013	28.810951	607
494	2	JZW-1	110.252986	28.81097	601
495	2	JZW-1	110.253121	28.810998	613
496	2	JZW-1	110.253121	28.81099	612
497	2	JZW-1	110.253175	28.811092	609
498	2	JZW-1	110.253443	28.811116	613
499	2	JZW-1	110.253604	28.811116	605
500	2	JZW-1	110.253631	28.811163	603

Code	Plot	Position	longitude	latitude	Altitude(m)
501	2	JZW-1	110.25398	28.811092	597
502	2	JZW-1	110.255135	28.810716	574
503	2	JZW-1	110.255243	28.810669	568
504	2	JZW-1	110.255323	28.810598	550
505	2	JZW-1	110.256049	28.81041	533
506	4	JZW-3	110.256908	28.80808	519
507	4	JZW-3	110.256908	28.80808	516
508	4	JZW-3	110.256425	28.80808	526
509	4	JZW-3	110.255699	28.807938	543
510	4	JZW-3	110.255565	28.807915	549
511	4	JZW-3	110.255565	28.807915	546
512	4	JZW-3	110.25535	28.807915	555
513	4	JZW-3	110.255377	28.807915	553
514	4	JZW-3	110.25527	28.807985	553
515	4	JZW-3	110.254921	28.807891	577
516	4	JZW-3	110.254894	28.807868	579
517	4	JZW-3	110.254894	28.807868	579
518	4	JZW-3	110.254706	28.807868	572
519	4	JZW-3	110.254706	28.807821	569
520	4	JZW-3	110.254652	28.807844	563
521	4	JZW-3	110.254652	28.807844	572
522	4	JZW-3	110.254437	28.807868	573
523	4	JZW-3	110.254437	28.807821	567
524	4	JZW-3	110.254168	28.807844	588
525	4	JZW-3	110.254115	28.80775	588
527	4	JZW-3	110.253363	28.807585	609
528	4	JZW-3	110.253148	28.807562	608
529	4	JZW-3	110.253443	28.807632	604
530	4	JZW-3	110.253094	28.807632	608
531	4	JZW3	110.25296	28.807656	609
532	4	JZW-3	110.25296	28.807656	608
533	4	JZW-3	110.252933	28.807679	612
534	4	JZW-3	110.25296	28.807656	612
535	4	JZW-3	110.252879	28.807656	615
536	4	JZW-3	110.252718	28.807632	620
537	4	JZW-3	110.252691	28.80775	621
538	4	JZW-3	110.252745	28.807726	611
539	4	JZW-3	110.25261	28.80775	613
540	4	JZW-3	110.252208	28.807774	612
541	4	JZW-3	110.252208	28.80775	622

Code	Plot	Position	longitude	latitude	Altitude(m)
542	4	JZW-3	110.252154	28.807726	619
543	4	JZW-3	110.252019	28.807797	630
544	4	JZW-3	110.251885	28.807868	634
545	4	JZW-3	110.251778	28.807821	642
546	4	JZW-3	110.251697	28.807726	650
547	4	JZW-3	110.25167	28.807891	644
548	4	JZW-3	110.251697	28.807938	651
549	4	JZW-3	110.251563	28.807962	655
550	4	JZW-3	110.25124	28.807915	659
551	4	JZW-3	110.25124	28.807915	653
552	4	JZW-3	110.251267	28.807962	656
553	4	JZW-3	110.25124	28.807962	652
554	4	JZW-3	110.250999	28.80815	662
555	4	JZW-3	110.250999	28.808197	659
556	4	JZW-3	110.250838	28.808174	664
557	4	JZW-3	110.250784	28.808197	665
558	4	JZW-3	110.250596	28.808221	606
559	2	JZW-1	110.248554	28.809421	717
560	2	JZW-1	110.247829	28.810645	756
561	2	JZW-1	110.247775	28.810692	751
562	2	JZW-1	110.247829	28.810645	750
563	2	JZW-1	110.247775	28.810692	750
564	2	JZW-1	110.247829	28.810645	749
565	2	JZW-1	110.247775	28.810692	749
566	2	JZW-1	110.24756	28.811116	754
567	2	JZW-1	110.247829	28.811728	760
568	2	JZW-1	110.247883	28.811822	762
569	2	JZW-1	110.24799	28.81187	764
570	2	JZW-1	110.248044	28.811916	759
571	2	JZW-1	110.248151	28.811916	765
572	2	JZW-1	110.248474	28.81201	752
573	2	JZW-1	110.250085	28.810127	727
574	2	JZW-1	110.2503	28.810221	698
575	2	JZW-1	110.250408	28.810221	684
576	3	JZW-2	110.259756	28.813516	477
577	3	JZW-2	110.259756	28.813516	497
579	3	JZW-2	110.259541	28.813611	505
580	3	JZW-2	110.259541	28.813611	501

Table S2.
Location and number of trees sampled for 9 populations in provenance.

Pop	Na	Ne	I	Ho	Fp	He	uHe	F
CTY	7	2.870	1.174	0.497	0.533	0.594	0.598	0.164
JZW-1	6	2.755	1.179	0.452	0.000	0.599	0.604	0.240
JZW-2	7	3.070	1.244	0.455	0.200	0.622	0.625	0.244
JZW-3	8	3.183	1.294	0.497	0.200	0.636	0.639	0.205
LYP-1	5	3.272	1.241	0.452	0.133	0.637	0.647	0.257
LYP-2	4	2.463	1.015	0.442	0.000	0.536	0.554	0.173
LYP-3	4	2.452	1.013	0.416	0.000	0.538	0.554	0.220
LYP-4	4	2.453	0.997	0.506	0.067	0.524	0.534	0.035
XNC	5	2.792	1.108	0.465	0.000	0.574	0.579	0.181
Mean		2.812	1.141	0.465	0.126	0.584	0.593	0.191

Na: number of different alleles; Ne: number of effective alleles; I: Shannon's Information Index; Ho: observed heterozygosity; He: expected heterozygosity with populations; uHe: unbiased expected heterozygosity with populations; F: fixation Index; Fp: no. private alleles (no. of alleles unique to a single population).

Table S3.
Genetic diversity parameters of 9 populations of Chinese fir. All values were multilocus estimates based on 15 microsatellite loci.

	CTY	JZW-1	JZW-2	JZW-3	LYP-1	LYP-2	LYP-3	LYP-4	XNC
CTY		0.088	0.069	0.038	0.057	0.093	0.081	0.080	0.052
JZW-1	0.092		0.014	0.025	0.013	0.067	0.064	0.069	0.071
JZW-2	0.072	0.018		0.018	0.004	0.051	0.039	0.049	0.037
JZW-3	0.041	0.029	0.020		0.010	0.051	0.038	0.044	0.024
LYP-1	0.063	0.021	0.010	0.016		0.045	0.034	0.041	0.043
LYP-2	0.104	0.080	0.062	0.062	0.060		0.006	0.026	0.063
LYP-3	0.091	0.077	0.050	0.048	0.049	0.025		0.016	0.049
LYP-4	0.087	0.077	0.055	0.051	0.051	0.040	0.030		0.059
XNC	0.057	0.076	0.041	0.027	0.051	0.076	0.061	0.066	

Table S4.
Pair-wise estimates of genetic differentiation between Chinese fir populations using Fst and Gst coefficients based on 15 SSR markers.

	CTY	JZW-1	JZW-2	JZW-3	LYP-1	LYP-2	LYP-3	LYP-4
JZW-1	0.357							
JZW-2	0.275	0.059						
JZW-3	0.144	0.101	0.073					
LYP-1	0.242	0.071	0.035	0.059				
LYP-2	0.356	0.255	0.197	0.201	0.192			
LYP-3	0.302	0.244	0.152	0.148	0.151	0.062		
LYP-4	0.273	0.236	0.164	0.150	0.153	0.099	0.073	
XNC	0.184	0.266	0.136	0.088	0.177	0.228	0.176	0.188

Table S5.
Genetic distance between the different population.

Author details

Ninghua Zhu[1], Xiaowei Yang[1]*, Zhiqiang Han[1] and Xiao Can[2]

1 Central South University of Forestry Science and Technology, Changsha, China

2 Jiangxi Environmental Engineering Vocational College, Ganzhou, China

*Address all correspondence to: 1498105349@qq.com

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Chapter 4

Conifers: Species Diversity and Improvement Status in Kenya

Joram M.E. Mbinga, Stephen F. Omondi
and Alice A. Onyango

Abstract

A wide range of exotic conifer species have been successfully introduced in Kenya since 1910 for the purpose of supplying wood, mainly for timber, pulp, and plywood industries. Among the conifers introduced, *Cupressus lusitanica* and *Pinus patula* have adapted well to local growing conditions and are now the key species widely planted in commercial plantations. The other conifer species are planted at secondary level or as ornamentals. In order to increase productivity, the key conifer species have been subjected to genetic improvement through selection, breeding, and hybridization. Results of tree improvement work on *C. lusitanica* and *P. patula* showed growth and productivity increase from 20 to 25 m^3/ha/yr. for *C. lusitanica* and from 25 to 30 m^3/ha/yr. for *P. patula*. Scaling up conifer plantations using the tree improvement technologies drawn for the two species is one of the strategies for closing the annual wood supply–demand deficit which is currently estimated at 10.3 million m^3. It is also one of the strategies for achieving 10% tree cover which is currently at 7.2%. The strategy encompasses the application of principles of tree breeding, improved germplasm, silviculture, pests and disease control. This presentation is a review of the status of conifer species since their introduction in Kenya.

Keywords: exotics, indigenous, tree improvement, growth, productivity, breeding, germplasm, silviculture, pests and diseases

1. Introduction

1.1 Background information

The entry of exotic conifer species in Kenya's forest development dates back to 1910's when the government made deliberate policy decision to put the forest sector under organised management [1]. Many conifer species were introduced and tested for their adaptability and growth under plantation conditions. Those species that showed faster growth and good wood and stem quality traits compared to the slower growing indigenous species were incorporated into plantation development programmes. To-date, about 80% of the 186,000 ha state forest plantation estate is composed of the two conifer species, *Cupressus lusitanica* Miller and *Pinus patula* Schiede [2]. They are grown in agriculturally high potential areas between 1500 and 2500 metres above sea level, with mean annual rainfall of between 1000 and 1750 mm. The main reasons for preference of the two species is their fast growth with a short rotation and economic importance in provision of timber for the construction industry, pulp for paper industry, plywood and fuelwood. They are also important in contributing to the country's efforts to raise tree cover from the present 7.2% to over 10% [3]. Also, their management regimes, silviculture, and other growth characteristics are already known because the species have been in the Kenyan landscape for a long time [1].

In order to make growing of conifers attractive to investors, strategies were drawn to increase the yield both in quantity and quality of timber and other tree products. This therefore called for formulation of tree improvement programmes that combines principles of tree breeding, silvicultural management and species-site matching. The programme would ensure a sustainable supply of improved seeds for establishment of highly productive commercial plantations. The first component of the tree improvement strategy was to continue with further introductions and evaluation of conifer species with potential for fast growth and adaptability. The second component was to work within the populations of the species that were already adopted for planting in commercial plantations and identify genotypes that are high yielding for further development. Resistance and tolerance to pests and diseases was among the considerations for species, provenance and genotype suitability. In this regard, the planting of *Cupressus macrocarpa* Hartweg and *Pinus radiata* D. Don, species were discontinued in Kenya from plantation development programme due to their susceptibility to pests and disease [4]. Tree improvement programme is also one of the strategies for addressing the deficit of wood supply, which is currently estimated at 10.3 million m^3 per annum [3]. Over time, the improvement programs have been reviewed and some species such as *Pinus tecunumanii* Eguiluz and J.P. Perry, *Pinus maximinoi* H.E. More, Pine hybrids, in addition to broadleaved non-conifer species have been considered to widen the choices of plantation species in the country [5, 6].

1.2 Introduction of conifers in Kenya

All conifer species that have been introduced to Kenya over the years fall into three families, namely, *Cupressaceae*, *Pinaceae*, and *Araucaraceae*. With the exception of the indigenous *Juniperus procera*, the rest of the conifer species listed in **Table 1**, below, were introduced by testing the species and or their provenances for growth and adaptability as candidate species for commercial plantations. Only two of the 42 listed species are planted on commercial scale, while 39 other conifers are planted on smaller scale as secondary species. This is because initial trials showed them to be either slow growing, have poor timber quality traits or are susceptible to pests and diseases compared to the two key conifer species. Several of these secondary species are planted within residences, in urban centres and along highways as ornamentals while others are still at species and provenance evaluation stage in research plots to determine their growth and adaptation to local growing conditions. The only indigenous conifer species, *J. procera*, is found growing naturally in the Western rainforest and the high-altitude Montane Forest types in the country. It has also been tried in plantations but found to be slower growing compared to the exotic conifers. It attains a productivity of Mean Annual Increment (MAI) of 3.73 m^3/ha/yr [9, 10].

The main objective of introducing conifers in Kenya was to supply wood for timber, paper and plywood industries. This was to take advantage of the fast growth of the conifers and the fairly matching climatic conditions in Kenya with that of their native countries. To maximise on the productivity of the conifers, there was formulated a conifer improvement programme whose ultimate goal was to develop high yielding varieties of priority conifer species for industrial plantations. The key outcome of the programme was to establish improved seed sources to enhance planting and ensure

Family	Species from the family	Status of species in Kenya
Cupressaceae	1. *Cupressus lusitanica* Miller	Most widely planted in commercial plantations
	2. *C. macrocarpa* Hartweg	Susceptible to disease
	3. *C. pyramidalis* O. Targ.Tozz	Ornamental
	4. *C. benthamii* Endlicher	Ornamental
	5. *C. torulosa* D. Don	Slow growth
	6. *C. sempervirens* L.	Slow growth
	7. *C. arizonica* Greene	Slow growth
	8. *Juniperus procera* Hochstetter	Indigenous, slow growth
	9. *Thuja occidentalis* Linnaeus	Ornamental
	10. *Cryptomeria japonica* D. Don	Slow growth
	11. *Callitris hugelii* Carrière	Drought resistant
	12. *C. preissii* Miquel	Drought resistant
	13. *C. calcarata* Silba	Drought resistant
	14. *C. rhomboidea* R. Brown	Poor quality poles and posts
Pinaceae	1. *Pinus patula* Schiede	Widely planted in commercial plantations
	2. *P. radiata* D. Don	Susceptible to disease
	3. *P. caribaea* Morelet	Lowland, secondary planting
	4. *P. oocarpa* Schiede	Experimental planting
	5. *P. pinaster* Aiton	Slow growth
	6. *P. kesiya* Royle	Slow growth
	7. *P. maximinoi* H.E. Moore	Experimental planting
	8. *P. tecunumanii* Eguiluz and J.P. Perry	Experimental planting
	9. P. hybrids	Experimental planting
	10. *P. taeda* Linnaeus	Slow growth
	11. *P. canariensis* C. Smith	Slow growth
	12. *P. elliottii* Engelmann	Secondary planting
	13. *P. strobus* Linnaeus	Slow growth
	14. *P. insignis* Douglas	Slow growth
	15. *P. halapensis* Mill.	Slow growth
	16. *P. pseudostrobus* Lindley	Slow growth
	17. *P. massoniana* Lambert	Slow growth
	18. *P. oaxacana* Mirov	Slow growth
	19. *P. montezumae* Lambert	Slow growth
	20. *P. michoacana* Martínez	Slow growth
	21. *P. pringlei* Shaw	Slow growth
	22. *P. ponderosa* Douglas	Slow growth
	23. *P. greggii* Engelmann	Slow growth
	24. *P. ayacahuite* Ehrenberg	Slow growth
Araucariacea	1. *Araucaria angustifolia* (Bertol.) Kuntze	Poor survival, slow growth
	2. *A. cunninghamii* Aiton	Poor survival, slow growth
	3. *A. heterophylla* (Salisb.) Franco	Ornamental
	4. *Agathis lanceolata* Lindley	In some homesteads

Source: adopted from [7, 8].

Table 1.
The diversity of conifer species introduced and tested in Kenya.

higher productivity of conifer plantation species [11]. The objectives of this review is to give the status of the introduction and genetic improvement of conifer species in Kenya, in particular the development of *C. lusitanica*, *P. patula* and *P. radiata*.

2. Genetic improvement of conifers in Kenya

The process of tree improvement is long-term. It starts with drawing a strategy which defines the scope in terms of the species of priority, the diversity within the populations of the species, and setting objectives of the programme. This is followed by field activities which involve selection of superior trees in the population, multiplication of the selected trees (genotypes), controlled pollination, hybridization, and genetic testing. Propagation of the selected superior genotypes and establishment of seed orchards is a key operation in the process [12]. These steps were followed in the programme to improve the two key conifer species in Kenya. However, since the whole process takes a long time before high quality seeds become available, a supplementary component of the programme ensures seeds for planting are available before the seed orchards from the main programme become productive. This involves the use of general forest stands that are inspected and certified to have a high proportion of phenotypically good trees to be used as sources of seed for expansion of plantations. In such cases, seed collection is confined to the trees that are bigger and more straight since the end market for these species is mainly sawn timber [11].

The method used to identify and select superior mother trees is based on visual observation followed by assessment of morphological features of the potential mother tree which has to be the most outstanding in comparison with the other trees in its neighbourhood. Total tree height and diameter at breast height (Dbh), are the main measurable traits for assessing growth of trees. Other quality traits such as tree form and stem straightness are also considered in such multi-trait selection [13]. Silvicultural and management operations to enhance seeding include thinning to obtain adequate spacing of trees in a stand. Vegetative propagation techniques and controlled pollination are often used to raise material for establishing seed orchards. In most cases, progeny tests are done in order to use the progeny trial data to assist in rogueing out poor performing mother trees in the seed orchards [14]. Further activities involve continuous infusion of new germplasm from other countries and broadening of the genetic base of the commercially prioritised tree species. New germplasm of species such as *P. tecunumanii*, *P. maximinoi* and pine hybrids have been introduced and are undergoing evaluation tests [5, 15].

2.1 Improvement status of the main conifer species

2.1.1 C. lusitanica improvement

Cupressus lusitanica (Mexican cypress) is native to Mexico but was introduced in Kenya in 1910 mainly from South Africa and France. It has since become an important industrial plantation species in high elevation areas between 1500 to 2500 m above sea level and annual rainfall of between 1000 and 1750 mm [16]. It is planted on commercial scale for production of sawn timber, plywood, pulp and poles for building and construction. It also provides services such as live fence, shade and ornamental [2, 17, 18].

Improvement of *C. lusitanica* has been carried out mainly to meet the requirements of sawn timber market. Therefore, the improvement objective was to raise productivity of volume of wood, and quality of tree stems. Mother tree selection was based on traits for rapid growth, stem form and shape, light branching and resistance to pests and diseases. Over the years, the programme progressively selected 453 outstanding *C. lusitanica* mother trees from commercial plantations. Those mother trees formed the base for improvement work. The mother trees are a source of propagation material and are multiplied through grafting, cuttings and progeny seeds which are used to establish seed orchards (**Table 2**). The breeding strategy for *C. lusitanica* has since been revised to incorporate recurrent selection of mother trees in progeny trials of subsequent generations [12].

Recent selections from F1 generation of progeny trials and from plantations has been carried out to yield mother trees to be used in establishing more seed orchards, further progeny trials, and expanded commercial plantations [12, 21].

Generally, it has been shown that plantations raised from seeds collected from seed orchards has led to improved performance with the crop attaining a mean height and diameter at breast height of 25.8 m and 30 cm, respectively, at sawn timber rotation of 30 years [12]. Other levels of height growth achieved at different ages are as shown in **Table 3** below [12]. Results from several progeny trials [4, 12, 19] show high heritability and genetic gains of key traits required in timber production. For example, heritability for diameter, height and volume was 0.89, 0.77 and 0.89, respectively, at 25 years while the genetic gain estimations was 7%, 6% and 12% for height, diameter and volume respectively [12]. Genetic improvement programmes elsewhere have shown that substantial gains were achieved through selection of superior trees for improved productivity of plantations and quality of products [14]. In terms of productivity, assessment of the F1 progeny trials yielded data which showed MAI improved from 20 to 25 m^3/ha/yr. [2, 22, 23]. The data was used to select another set of plus trees to form the F2 generation that is currently being tested in progeny trials. The F2 seed orchards similar to **Figure 1**, below, are in the process of being established. Demand for improved cypress planting materials (seeds and seedlings) has been increasing hence the need for more seed orchards and further genetic improvement of the germplasm [24].

2.1.2 Pinus patula and pine hybrids improvement

Pines are fast growing exotic softwood tree species introduced to Kenya from South Africa (Ex-Mexico) in early 1920's to provide raw material for the rapidly expanding wood-based industry. Many pine species were also introduced and planted in species site matching trials in the 1940s to determine their adaptability and growth patterns [25]. From these trials, *P. patula* and *P. radiata* were initially

Species	No. of seed orchards	Total area (ha)	Annual seed production (kg)
C. lusitanica	22	39.5	550

Source: adopted from [19, 20].

Table 2.
Seed production status of C. lusitanica.

Age (yrs)	1	3	5	8	10	15	20	25	30
Mean Ht (m)	0.9	3.9	7.0	11.5	13.3	18.2	21.2	23.6	25.8

Source: adapted from [12].

Table 3.
Mean height growth of improved C. lusitanica on average sites in Kenya.

Figure 1.
(Left) A seeding 12-year-old C. lusitanica seed orchard with (right) a zoomed in branch, at Londiani, Kenya. Source: Photograph by Mbinga J.

selected for large-scale planting due to their fast growth and their relative ease of establishment. However, *P. radiata* was subsequently discontinued from plantation establishment due to its susceptibility to *Dothistroma pinii* fungal disease. Currently, *Pinus patula* is the main pine species being planted on commercial scale. It grows well in areas where elevation ranges from 1500 m to 2500 m a.s.l, with annual rainfall above 1000 mm., predominantly in Central Kenya and parts of Rift valley. Its silvics is well developed where it is planted at a spacing of 2.75 x 2.75 m and 2.5 x 2.5 m for pulpwood and sawn timber cycles respectively. There is no thinning for pulpwood cycle, but for timber, the plantations are thinned from the initial density of 1600 stems per ha to 800, 600, and 356 stems per ha at ages of 10, 18, and 25 years respectively [8]. The primary market for *P. patula* is sawn timber, paper and plywood. The species has undergone improvement through breeding with the objective of increasing wood volume productivity per unit area and stem quality. Main traits considered are fast growth, good form, light branching, and resistance to pest and disease. For breeding purposes, *P. patula* heritability for various traits were found to be as follows; 0.44, 0.82 and 0.40 for height, diameter and stem form respectively [26]. Therefore, selections of best performers based on the trait of diameter at breast height will give significant gains since the trait has a high heritability. *P. patula* is also used as a main hybrid parent where it is crossed with many other pines to produce pine hybrids. The strategy guiding the improvement of *P. patula* [12] was revised to provide for mass selection of superior mother trees from existing plantations and using them to establish clonal seed orchards (**Table 4**), (**Figure 2**). The performance of the selected mother trees were tested through progeny trials. Over the years, the cumulative number of breeding mother trees selected for this species are 464 trees which forms the base for further improvement work [12].

Species	No. of seed orchards	Total Area (ha)	Annual seed production (kg)
P. patula	16	62.5	380

Source: adopted from [19, 20].

Table 4.
Seed production status of P. patula.

Figure 2.
A six year grafted P. patula clonal seed orchard at Kamara, Kenya. Source: Photograph by Mbinga J.

The plus trees were also conserved in a clonal Tree Bank at Muguga [27], and their progenies used to establish progeny trials. The programme set target of raising its productivity from 25 m^3/ha/yr. to 30 m^3/ha/yr., thereby making genetic gains in the traits under consideration [12, 22]. The gains have contributed to increases of 10%, 5%, and 11% in height, diameter at breast height and volume production per hectare respectively [6, 28].

In the hybridization component, *P. patula* has been crossed with other pines to produce hybrids some of which perform better than parent trees while others are more adaptable to different growing conditions. Kenya Forestry Research Institute collaborates with the Conifer Improvement and Conservation (CAMCORE) programme, based in North Caroline, United States of America, and through this initiative, several pines and pine hybrid germplasm have been accessed and are being tested to determine their performance.

Preliminary results of hybrid trials indicate that hybrids of *P. patula* and *P. tecunumanii*, (Low elevation provenance), outperforms all the other hybrids, as listed in **Table 5**, and the pure *P. patula* control with respect to survival, height, and diameter at breast height. New germplasm of *P. maximinoi* and *P. tecunumanii* were also showing good growth and adaptability at Turbo site in western Kenya.

2.1.3 Improvement of Pinus radiata in Kenya

In Kenya, *P. radiata* was the most favoured species for pulp and paper products. It has good pulp and paper qualities, produces higher volume yield per unit area, has higher wood density, longer and narrow tracheids, low lignin content and less extractives. However, the species is highly susceptible to *D. pinii* fungal disease that led to discontinuation as a plantation species in Kenya [29]. Efforts to control the fungal disease were initiated in 1970s through breeding for disease resistance where selection of trees on the basis of lower severity of disease symptoms was done and supplemented with infusion of more progenies from Australia and New Zealand. Such trees were propagated into seed orchards and progeny trials to test the persistence of their observed tolerance to *D. pinii* disease. The trials were established in areas that are prone to *D. pinii* fungal infestation namely: Timboroa, Nabkoi and Kaptagat [30, 31].

S/no.	Hybrid	Survival %	Height (m)	Dbh (cm)
1	*Elliotii* x *tecunumanii*, High elevation	43	1.55	2.31
2	*Elliotii* x *taeda*	73	3.15	5.00
3	*Patula* x *greggii*	65	4.33	5.34
4	*Patula* x *taeda*	47	4.37	6.36
5	*Elliotii* x *caribaea*	78	4.44	7.36
6	*Caribaea* x *tecunumanii* Low elevation	70	4.72	7.13
7	*Patula* x elliotii	39	5.18	7.01
8	*P. patula* Pure spp	60	5.41	6.99
9	*Tecunumanii*, Low elevation x *caribeae*	82	5.43	7.64
10	*Tecunumanii*, High elevation x *oocarpa*	76	5.52	7.39
11	*Patula* x *tecunumanii*, High elevation	63	5.77	7.74
12	*Patula* x *oocarpa*	69	6.06	8.43
13	*Patula* x *tecunumanii*, Low elevation	85	6.49	8.69

Source: [6].

Table 5.
Summary statistics for pine hybrids at Turbo, Kenya at age 4 years.

Results from trials established using material imported from New Zealand show the 28-year-old trial at Timboroa, Kenya had the best growth with an MAI productivity of 40 m^3 /ha/yr. and mean Dothistroma disease score of 2, which translates to susceptibility level of 25-50% (**Table 6**).

To-date, the new *P. radiata* families infused from New Zealand, as well as the local selections forms the base population of the species. Since 75% levels of disease resistance or tolerance showing a mean disease score of 1 [31], has not been achieved, the genetic material present have been conserved in conservation plots to provide opportunities for future improvement [30]. Lessons learnt from the experience of breeding for disease and pest tolerance such as in *P. radiata* indicates that gains can be small and take long to be realized. also the future of plantation forestry

Site	Source of material	Age (yrs)	Mean ht (m)	Mean Dbh (cm)	Mean disease score (scale 1-5) Where; 1=least susceptible 5=most susceptible	Mean volume (m^3/ tree)	Productivity MAI m^3/ha/ yr
Nabkoi Compt. 9	New Zealand	29	29.3	30.4	2	1.24	21
Timboroa compt. 2 J	New Zealand	28	33.0	39.4	2	2.25	40
Kaptagat Compt.1Y	Local selections	24	28.9	33.5	2	1.37	29
(comparison) *P. patula*	In general plantations	30	30.0	34.5	—	1.8	30

Source: [31].

Table 6.
Growth and productivity of Pinus radiata progenies in areas with prevalence of Dothistroma needle blight disease in Kenya.

requires a diversity of species with populations having a broad genetic base in order to safeguard against negative effects of pests and diseases.

3. Conclusions

Among the conifer species introduced in Kenya to supply timber for construction, pulpwood and plywood industries, two of them have performed well enough to meet the intended purpose. The two species, *C. lusitanica* and *P. patula*, are currently the most widely planted and dominate the 186,000 ha of public forest plantations. The other less successful conifers are planted at secondary level. Tree improvement work on the key conifer species has been done using the classical approach of selections, testing and propagation. Even though this approach is slow, it ensured continuous supply of improved seeds that yield timber trees of a quality that meets market requirements. Continuous improvement work focusing on pine hybrids promises to sustain higher timber productivity in traditional plantation areas and have potential to grow in marginal sites thereby expanding the area available for commercial forestry as well as an adaption to climate change. For the future, it may be necessary to hasten tree improvement process by reviewing the classical approach and adopting modern molecular tools and biotechnology to meet tree improvement objectives faster.

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Author details

Joram M.E. Mbinga*, Stephen F. Omondi and Alice A. Onyango
Kenya Forestry Research Institute, Nairobi, Kenya

*Address all correspondence to: jmbinga@kefri.org; jmbinga@gmail.com

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Chapter 5

Larch: A Promising Deciduous Conifer as an Eco-Environmental Resource

Laiye Qu, Yannan Wang, Oxana Masyagina, Satoshi Kitaoka,
Saki Fujita, Kazuhito Kita, Anatoly Prokushkin
and Takayoshi Koike

Abstract

Larch species are widely distributed in the northern hemisphere where permafrost and seasonal frozen soil exist. This species with heterophyllous shoots has been intensively planted in northeast Asia as well as in northeast China as the principal afforestation species for restoring agricultural lands to forests from 1999. Although approximately 15 species exist in the northern hemisphere and they are easy to hybridize. Among them, Japanese larch grows the fastest and was exported to Europe as a breeding species from early 20s. Although Japanese larch is tolerant to cold, it suffered from various biological stresses. After nearly 40 years of vigorous breeding effort, hybrid larch F_1 (Dahurian larch × Japanese one) was developed with simple propagation methods. With the use of free-air CO_2 enriched (FACE) systems, we revealed growth responses of the F_1 and its parent larches to environmental conditions. From experiments, F_1 showed high responses to elevated CO_2 and O_3 but not so much to N loading. As future perspectives for larch plantations as an important eco-environmental resource, we expect to afforest F_1 seedlings infected with ectomycorrhizae (e.g., *Suillus* sp.) for efficient afforestation at nutrient-poor sites and at the same time for the production of delicious mushrooms.

Keywords: larch, hybrid, heterophyllous shoot, growth, changing environment

1. Introduction

The larch species are a typical light-demanding deciduous conifer, ectomycorrhizal (ECM) tree species, and dominant in the northern hemisphere [1, 2]. Among genus *Larix*, Dahurian larch (*Larix gmelinii*; including *L. cajanderi*) is especially dominating permafrost ecosystems has an essential role in climate change in the Far East of Eurasia [3–5]. If we would follow the idea of the well-known Köppen [6] and Whittaker [7], "estimated" vegetation at Far East Russia and northeast (NE) China should be a type of steppe. However, actual vegetation there is light-Taiga (dominating Dahurian larch) due to the existence of permafrost [2, 3]. In this chapter, we discuss the environmental role of larch forests and global climate change.

Some larch species are typical afforestation species in NE China, Russian Far East, Korea, and Japan. Most larches can tolerate cold and late frost [8], thus attaining significant biomass with a high growth rate in cold regions [2, 3]. Due to these good growth traits, larch (Japanese larch: *Larix kaempferi*: syn. *Larix leptolepis*) was exported to Europe as a pollen resource. Also, Japanese larch had intensively planted in the Korean peninsula and NE China; these forests are used for timber production. From 1999, the Chinese government decided to reforest farmland (<25° slope) and degraded area (i.e., Natural Forest Conservation Program [NFCP]) and employed Dahurian larch (*L. gmelinii*) in NE China to increase forests [9]. From physiology and genetics to ecological point of view where the larch species will contribute as a resource of the sustainable developmental goals (SDGs).

The physical environment surrounding the biosphere has been dramatically changing worldwide. Especially, atmospheric CO_2 concentration ($[CO_2]$), nitrogen (N) deposition, and ground-level atmospheric ozone concentration (O_3) have increased rapidly since the Industrial Revolution [10, 11]. Furthermore, these physical environmental changes will become serious in the near future because of increased energy demands due to rapid economic growth, industrialization, and urbanization in Asian countries. For sustainable use and adequate management of forest resources, we must therefore clarify the response of trees to these environmental changes.

Nowadays, larch trees are intensively planted and lumbered not only in northern Japan [12, 13] but more widely in the northern Eurasian continent. However, knowledge about the susceptibility of this species to environmental stresses is still limited, except for biological stresses, for example, shoot blight and root rot disease [14], and physical stresses, that is, low temperature [8]. Will larches maintain their high growth rate and extensive establishment under the changing environment? Recently, several researchers have studied effects of environmental changes on larch species [2, 4, 13, 15]. The information will be useful for sustainable use and adequate management of larch plantations. In this chapter, we integrate previous studies examining the growth and ecophysiological responses of larch species including their hybrid to environmental changes, and propose the future direction for utilization of larch species.

2. Botanical traits

2.1 Larch species feature

Genus *Larix* is broadly distributed in the northern hemisphere and consists of 17 species including variety [16]. Among them, four species are dominant (**Figure 1**): Siberian larch (*Larix sibirica*) distributes from the Ural mountain to Lake Baikal, Dahurian larch (*L. gmelinii* var. *gmelinii*: syn. *L. dahurica*, partly including *L. cajanderi*) covers eastern parts of the Eurasian continent; mainly on Sakha Republic (Yakutia; Russia) and northeastern part of China; Mandsburica larch (*L. olgensis*) and Hokshi larch (*L. principis-rupprechtii*) are distributed there [3].

Other variety of Dahurian larch (*Larix gmelinii var. japonica*) distributes in the Kuril Islands, and in Japan around more than 10,000 years ago; currently, Japanese larch is naturally distributed in the central part of Japan and the northern limit is located Mt. Mano-kami at Northern Honshu Island [17, 18].

In central Europe, European larch (*L. decidua*) is widely distributed even in forming tree-line at the central Alps along with way of an avalanche. American larch (*Larix laricina*) distributed in North America [19, 20]. From 1900's days, Japanese larch had exported to Europe to increase growth rate and stress tolerance because larch species are easily formed by means of a hybrid produced by interspecific crosses [21, 22]. Details of larch species in China are referred to Section 3.2 (Y. N. Wang).

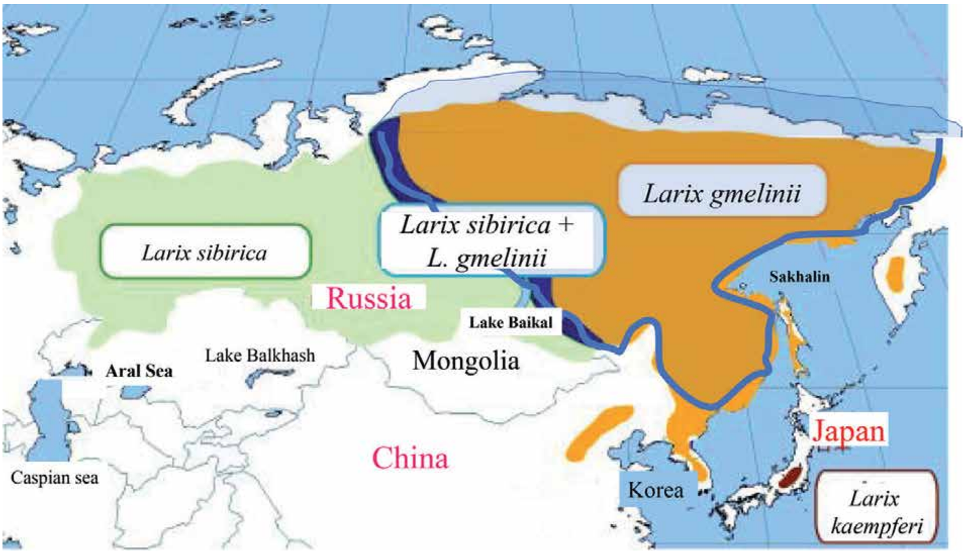

Figure 1.
Distribution of larch species in Eurasian continent and Far East Asia (illustrated from: Abaimov et al. [3], Larix gmelinii *including* L. cajanderi*).*

2.2 Water relations

Deciduous needle habit of larch species may contribute to the dominance of these species in permafrost regions as compared with an evergreen conifer (*Picea mariana*) in Alaska [23]. This deciduousness in leaf habit implies that larch can avoid severe water deficits (including winter desiccation damage) during early spring when soil is still frozen (**Figure 2**). Seedlings and lower branches of larches usually keep their overwintering needles until the xylem pressure potential is above −1.5 MPa (Koike unpublished data). In fact, winter desiccation damage in Sakhalin fir (*Abies sachalinensis*)

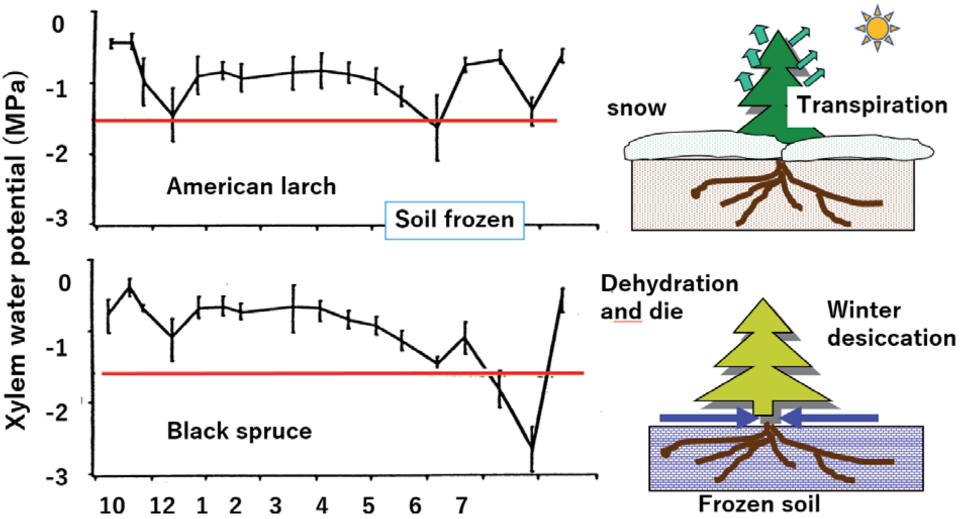

Figure 2.
Seasonal change in the water relation in American larch and Black spruce (left), schema of winter desiccation mechanism (right) [24]. American larch: deciduous, Black spruce: evergreen (Adaptation from: Berg and Chapin [23]).

frequently occurs in plantations facing the Pacific Ocean side in Japan where they have shallow snow depth Sakai [24].

2.3 Needle morphology and photosynthesis

The photosynthetic rate of larches is markedly higher than that of other conifers [25]. However, the initial slope of the light-photosynthetic curve for larch is gentler than that for several conifers, and has similar traits to the C_4 plant [26]. The possibility that the larch is a kind of C_4 plant was nevertheless disproven by a photosynthesis experiment using ^{14}C-labeled CO_2 [26]; larch is concluded as a C_3 type plant.

This high growth results from its high photosynthetic rate and unique arrangement of two different types of needles, that is, short-shoot and long-shoot needles [27]. To reveal the photosynthetic characteristics of short- and long-shoot needles of the sunny canopy of the larch trees *in situ* using a canopy tower, the seasonal change of gas exchange characteristics were measured accompanied by leaf mass per area (LMA), foliar nitrogen content (N) of the heterophyllous needles over 3 years. No marked difference in light-saturated photosynthetic rates (P_{sat}) was observed between short- and long-shoots after leaf maturation to yellowing, although the difference was found in a specific year (**Figure 3**), which only indicates that seasonal fluctuation in temperature and soil moisture determines the photosynthetic capacity of needles [27].

The large annual and seasonal variations in P_{sat} in both shoots were found to be mainly determined by climatic variations, while shoot types determined the strategy of their photosynthetic N utilization (N use efficiency, retranslocation, etc.) as well as the stomatal regulation as found in deciduous broadleaved tree saplings grown under larch forest [28].

Although there is no difference in the growth and development of seedlings of Japanese and hybrid larch F_1, the temperature dependence of photosynthesis in

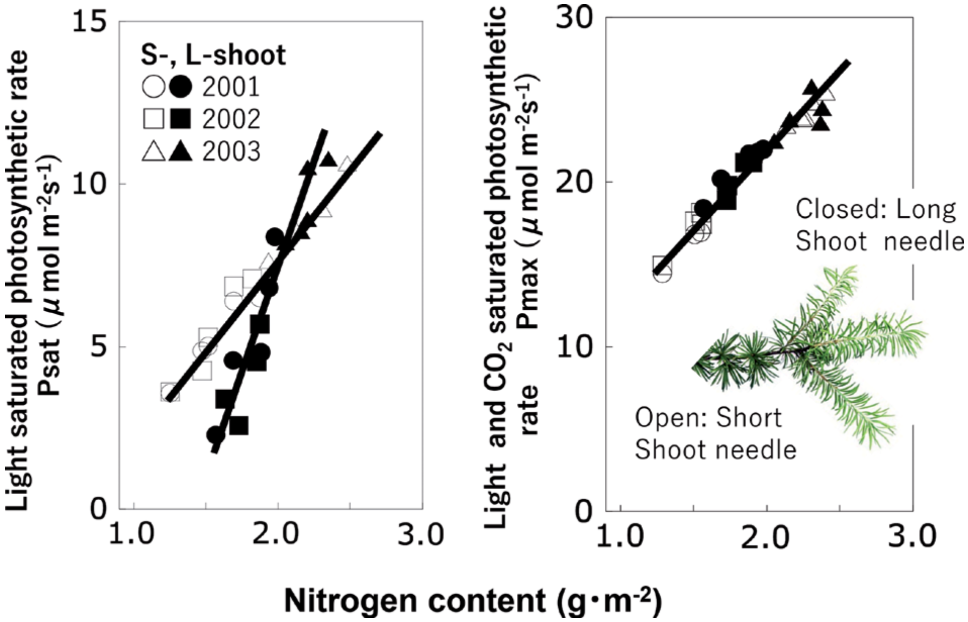

Figure 3.
Variation of light-saturated photosynthetic rate (P_{sat}) at ambient (left) in short- and long-shoot needles at larch canopy and no variation of P_{max} at CO_2 saturation (right) located at larch canopy in terms of N content, measured during 2001–2003 in situ (Adaptation from: Kitaoka et al. [27]).

hybrid larch shows greater photosynthetic starch accumulation capacity than in Japanese larch [29].

2.4 Photosynthates allocation: Individual scale

Carbon (C) allocation pattern of photosynthates may be essential for the growth and survival of plants [30]. Allocation of photosynthates to the root system in larch seedlings, for instance, can maintain growth at low soil pH [31]. Symbiotic microorganisms in larch root require 10–20% of photosynthates of host plants [32]. Larch seedings inoculated with commercial ectomycorrhiza: ECM (*Pisolithus arrhizus*: *Pa*) increased photosynthesis, which is more accelerated by a mixture of ECM collecting from the larch forest floor (**Figure 4**). From Farquhar et al. [33], A-Ci (intercellular CO_2 concentration: Ci and assimilation rate: A) indicates efficient use of photo-assimilate in ECM infected hybrid larch F_1. With an increasing number of infected ECM, stomatal limitation (Ls) decreased.

In fact, the growth of larch species is closely connected with the ubiquitous ectomycorrhizal fungal association. Symbiotic ECM improves nutrients (phosphorus, P; nitrogen, N) and water uptake, and buffers against environmental stress [34–38].

2.5 Individual to forest scale

Photosynthate allocation is essential not only for plant growth and survival but is also directly related to the photosynthetic productivity of forested stands. Photosynthetic production is a compromise between the instantaneous photosynthetic capacity of leaves and leaf longevity (e.g., [22, 34]). Photosynthetic production (biomass) is therefore tightly linked to leaf area index (LAI; leaf area per unit area; $m^2\ m^{-2}$). As a result, LAI has been studied in several terrestrial ecosystems. Larch forests have a relatively small LAI value of 4.1 [39] (**Table 1**). The aboveground production rate of larch in early autumn is estimated to be similar to that of evergreen conifers (e.g., *Picea abies*) in a cool-temperate environment.

2.6 Continental scale

As summarized by Osawa et al. [2], carbon (C)-allocation of permafrost ecosystem has unique characteristics and key of survival of larch on permafrost in Central and Far East Russia where a vast area of forest exists on continuous permafrost [45]. According to them, "Deciduous coniferous taiga, larch ecosystem is one of the unique biomes in northeastern Eurasian Continent, where a vast area of forest exists on continuous permafrost." We defined the active soil layer as the melted soil layer between the ground surface to the front of frozen soil. Based on the field survey, three representative sites were selected: (1) a forest near Yakutsk in Yakutian Basin, eastern Siberia (62 N–129E), (2) a forest near Tura in central Siberian Plateau (64 N–100E), and (3) a forest tundra transition near Chersky in Kolyma lowland (69 N–160E) [45]. C storage in these ecosystems was estimated in both aboveground and belowground biomass, in the forest floor, and in active layer as for soil organic C and as carbonate-carbon (**Figure 5**).

Matsuura et al. [45] well summarized that organic C in the soil in active layer was the largest component in the sites. Soils in Russia (Yakutsk and Tura) sites indicated carbonate-C accumulation in the active layer, which might result from an extreme continental climate with low annual precipitation of around 200–500 mm $year^{-1}$ and big temperature range C storage in above- and below-ground biomass varied among sites, however, ratios of above–/below-ground biomass C had a narrow range from 1.1 to 1.5. The high allocation rate of C to below-ground

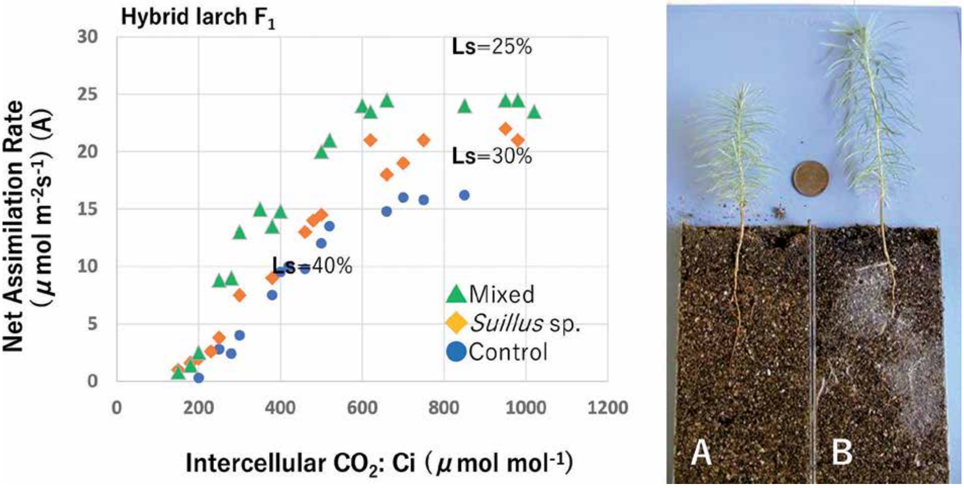

Figure 4.
Assimilation and intercellular CO_2 concentration (A-Ci) relation in hybrid larch F_1 inoculated with ECM (one species vs. multi-infection) and stomatal limitation (Ls %). Right: A: control; no infection of ECM, B: in infected with commercial ECM (Pisolithus arrhizus) (Adaptation from: Qu et al. [32]).

Forest type	Foliage mass (ton hm^{-2})	Leaf area index (LAI) (m m^{-2})	No. stands	References
Deciduous forests	2.9 ± 1.5	3.0–6.0	98	[40, 41]
Larch stand	2.9 ± 1.0	2.5–4.5	28	[41, 42]
Pine (red and black)	6.8 ± 1.8	3.5–6.0	60	[41, 42]
Evergreen forest	8.6 ± 2.6	5.5–9.0	46	[41, 43, 44]
Evergreen conifers	16.0 ± 4.5	5.0–10.0	49	[40, 42]
Cryptomeria (cedar)	19.4 ± 4.9	4.5–8.5	97	[42, 43]

Table 1.
Forest types, foliage mass, and LAI.

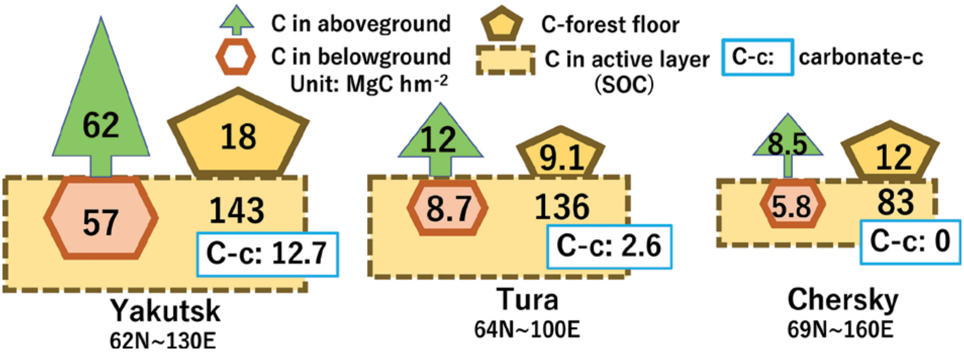

Figure 5.
Carbon storage and allocation in different larch ecosystems in eastern Eurasia (Adaptation from: Matsuura et al. [45]).

part resulted from a kind of adaptation to effective water and nutrient acquisition under nutrient-limited environment due to low soil temperature for litter decomposition [46].

3. Vegetation characteristics

3.1 Russian Far East and central Siberia

In this section, we should point out important evidence; "Permafrost layer nurtures light-Taiga and the canopy protects permafrost" [2], especially at Yakutia; northern Far East (FE) Russia. According to the classic idea of climatologists and community ecologists [6, 7], Yakutian vegetation should be steppe or grassland, however, light Taiga mainly composed of larch is well developed [47]. Even under the low precipitation of continental climate, the permafrost provides water from belowground to aboveground, which is accelerated by extra-harvesting, forest fires, global warming [2, 48], etc. However, the accumulation of salt (mainly of Natrium compounds) on the ground surface will inhibit forest regeneration [49]. Much worse, the emission of greenhouse gas (CO_2, CH_4, N_2O, etc.) and unknown microbes will increase from melted permafrost. As an old saying of Yakutian people, "we can make one grave per one." Alas (=pond appeared after harvest or fire in the Taiga)", which points to their method of sustainable forest management method (**Figure 6**).

Regeneration of larch is moderate and larch-dominated Taiga is recovered (canopy closure) 20 years after forest fires. This is attributed to an increase in depth of the active soil layer by heat from fires and/or charcoal accumulation. Another 80–100 years and more after the canopy closure, the closed canopy gradually becomes sparse because sunlight to the forest floor is intercepted and will recover the depth of the active soil layer. As a result, competition of aboveground may be caused by limited amount of water and nutrients, but not only by light resources [2, 48].

3.2 Distribution of larch in China (Y. N. Wang)

In China, there are two sections in genus *Larix*: Sect. *Larix* and Sect. *Multiseriales*, 11 species (with four endemic species, two introduced species) as shown in **Table 2** [53].

Figure 6.
The Alas developed after harvesting by local people of Yakutia (Adopted from: Koike [50], with permission).

Section	Species	Elevation (m)	District, location habitat	
Sect. *Larix*	*Larix gmelinii*	300–2800	Hebei, Heilongjiang, Northwestern Henan, Jilin, Nei Mongol, Shanxi (Daxing'anling, Xiaoxing'anling Mt.)	Rocky slopes, peatlands, swamps, lowland subarctic plains, river basins, valleys
	(*L. gmelinii* var. *principis-rupprechtii*)	600–2800	Hebei, Northwestern Henan, Shanxi	Usually on rocky slopes
	Larix olgensis	500–1800	Jilin, Eastern Liaoning	Mountains, moist slopes, swamps
	Larix sibirica	500–3500	Xinjiang, Altai M., Eastern Tianshan M., lowland taiga	Cold, relatively dry, long day-time during July to August
	Larix kaempferi		Hebei, Heilongjiang, Henan, Jiangxi, Jilin, Liaoning, Shandong	Introduced, cultivated
	Larix decidua		Jiangxi (Lu Shan), Liaoning	Introduced, cultivated
Sect. *Multiseriales*	*Larix griffithii*	3000–4100	Southern and Eastern Xizang	Mountains
	Larix speciose	2600–4000	Southeastern Xizang, Northwestern Yunnan	Mountains
	Larix kongboensis	3200–3500	Southeastern Xizang (Gongbo'gyamda)	Rocky slopes
	Larix mastersiana	2300–3500	Sichuan	Mountains
	Larix himalaica	3000–3500	Southern Xizang	River basins, valleys
	Larix potaninii	2500–4300 (−4600)	Southern Gansu, Southern Shaanxi, Sichuan, SE Xizang, Northern Yunnan	Mountains, river basins

Refs: Fang et al. [51], Li et al. [52], and Flora of China [53].

Table 2.
Larch distribution in China.

L. gmelinii are mainly distributed in the Daxing'anling, Xiaoxing'anling mountains in Northeast China, especially in the Daxing'anling mountains (**Figure 7**). It is the most representative species of cold temperate coniferous forest with stands occupying the large area with high biomass stocks.

It is the main wood production base in China and one of the main tree species for forest management, and artificial afforestation in Northeast China. With the thawing and shrinking of permafrost, the distribution of *Larix Xing'an* will gradually move northward, and the proportion of *L. Xing'an* in the ecosystem of *L. Xing'an* forest will also gradually decrease as suggested by Abaimov et al. [22]. According to the prediction of the distribution model of larch in Xing'an, the distribution of larch in the community of *L. Xing'an* forest will gradually move northward or even outward under the climate change in the future [52].

Larix principis-rupprechtii is a typical zonal-type tree in the middle and high mountains of North China. Its rapid growth, excellent resistance to cold and drought, and woody materials play an important role in an ecological component and strategic timber reserve in the mountainous areas of North China [51].
The natural distribution is mainly in Shanxi and Hebei provinces. With the promotion of cultivation technology, in recent years, its plantation area was

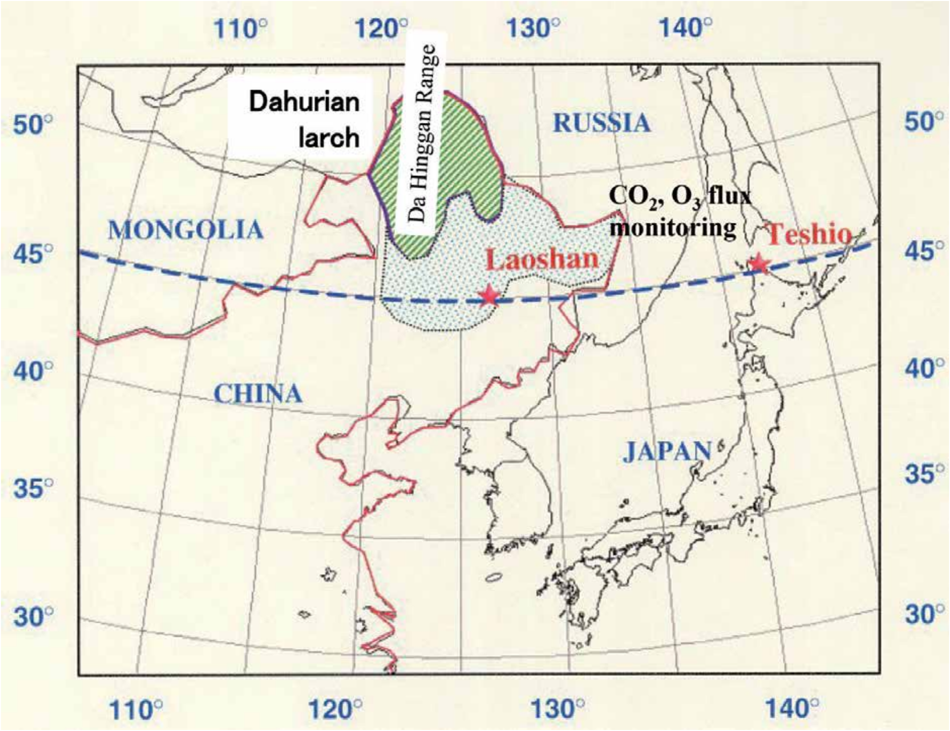

Figure 7.
*Distribution of Dahurian larch (*Larix gmelinii*) forest in NE China (adaptation from: Mao et al. [54], Wang et al. [55], and Shi et al. [46]).*

been widened to the low altitude areas of Shanxi and Hebei provinces, Inner Mongolia, Beijing, Shandong, Liaoning, Shaanxi, Gansu, Ningxia, Xinjiang, and other provinces and cities. Due to the great influence of geographical environment and natural conditions, as well as the influence of climate change, most of the introduction and cultivation in other areas except the original place are not good.

Japanese larch (*Larix kaempferi*) is native to Japan and was introduced to China in the late 19th century, which has a wide range of adaptation, rapid initial growth. It has become the main afforestation tree species in the mountains south of 45 N in the eastern northeast of China, mainly cultivated in NE China, North China, NE China, and SW China [53].

Larix sibirica is a coniferous species endemic to NW China, only distributed in a small amount in Xinjiang areas distributed in the northern part of NE China. It is generally distributed in cold mountainous areas or on the banks of low mountain valleys, is one of the most distributed building species in the Altai Mountains. It is mainly distributed on the slopes of wet airflow and the shady and semi-shady slopes of river valleys [53].

4. History of afforestation in Far East Asia and forest fires

Forests in Russian are mainly regulated by forest fires and are naturally regenerated [13, 16, 47]. The Chinese government has been intensively planting three species including larch from 1999 [9, 56]. In Japan, the establishment of plantations of Japanese larch was not successful due to several biological stresses, especially in Hokkaido island [13, 15].

4.1 Far East Russia and central Siberia

Larch forest conservation and silviculture in Far East Asia should be considered on the high pressure of forest fires [2, 3, 46, 57]. Forest fires have been regulating vegetation dynamics there, especially Russian Far East [47, 57]; the essential role of biochar is well evaluated (**Figure 8**) [58, 59].

4.2 China

Larch (*Larix* spp.) is one of the most representative forest component species in mountain and temperate zone under cold conditions, forming the northern coniferous forest with the largest area and the highest volume in the eastern part of the Eurasian continent. As native species are widely distributed in NE and north China, larch forests play a pivotal role in maintaining forest ecosystem functions and mitigation of carbon concentration in the atmosphere.

Larch is naturally distributed in mountain areas of NE China, Inner Mongolia, North China, and SW China. Due to its characteristics of cold resistance, fast growth, fine wood structure, and strong corrosion-resistance, it has become the main afforestation and fast-growing high-yield tree species in northern China [9]. Since the founding of the People's Republic of China, a large area of larch plantation has been built successively, which is an important reserve forest resource in China [60]. Larch usually forms a large area of the pure forest after forest fires [15, 46, 47, 57], or composition of larch-based mixed forest with birch, poplar, spruce, and other coniferous and broad-leaved trees [46].

According to the data of the 9th National Forest Inventory in China (2014–2018), the national forest coverage rate is 22.96%, with a forest area of 220 million hm^2, including 79.54 million hm^2 of the artificial forest, ranking first in the world [60].

Figure 8.
A view of burned larch forest after trunk fire at around Amur state, Russia (Photo courtesy by: Dr. Semyon Bryanin and Dr. Makoto Kobayashi).

According to the report of Global Forest Resources Assessment (FRA) in China, the growing stock in the forest of larch species reached about 1,200 million m^3 [56]. As dominant tree species components, the top three tree species are oak (*Quercus mongolica*) forest, Chinese fir (*Cunninghamia lanceolata*) forest, and larch forest. It can be seen that larch plays an important role in the forest composition of China [9].

As mentioned above, the Chinese government has been intensively planting three kinds of tree species (Dahurian larch for NE, Chinese fir for SW, and poplar for all parts) on farmlands and degraded areas. This project is called as NFCP, which emphasizes "expansion of natural forests and increasing the productivity of forest plantations" [9], and attained the largest new plantation area in the world [56, 60]. This area by 2019 is larger than the whole Japanese land area. On the occasion of the announcement of the leader Mr. Xi Jinping, one of the Chinese ecological policies orients us on how to conserve forest as an ecological unit. Based on this statement, the conservation of the forest ecosystem is one of the national key projects for "ecological culture city" [61]. However, NFCP proposed they would not harvest their own trees. In connection with this, Chinese trade in timber may strongly depend on forests in Amur state, Russia, and other states located in the opposite bank where no "border" between the two states due to the river frozen during winter.

4.3 Japan

In Japan, the establishment of larch plantations had been not successful due to several biological stresses, in Hokkaido island as well as a central part of Japan [39]. From silviculture records [53, 62], larch plantation started to use mountain stock in Nagano prefecture in central Japan during 1624–1645. At around 1890, the production method of larch seedlings has established in central Japan and had expanded to Hokkaido around 1910 [18, 53]. Intensive plantation of larch was intended to produce mine timber equipped with high compressive strength by short term rotation culture of around less than 30-year-old.

At the latter 1970's days, the outbreak of grazing damages on the needle by larch sawfly (*Pristiphora erichsonii*) spread around the southern part of Hokkaido and continued around mid-1980 [18]. The grazing by sawfly again started from 2000 and continues until now in whole Hokkaido Island (**Figure 9**). This may be due to recent dry and warm climatic conditions and big stresses caused by attacking by *Armillaria* sp. [18]. The responses of the Japanese larch (*Larix kaempferi*) to graze by the larch sawfly (*P. erichsonii*) were examined from the perspective of the carbon/nutrient balance (CNB) hypothesis [64]. The defoliation intensity was determined from canopy photos taken from 2009 to 2012 in seven Japanese larch plantations in central Hokkaido, Japan. A decrease in foliar nitrogen and increases in phenolics, tannins, and the CN ratio was found in the years following severe defoliation. The influence of defoliation was fluctuated over years. These results indicated that the past defoliation history additively affected the foliage properties in the 2 years following insect grazing. Phenolics and sugars did not increase linearly with the leaf CN ratio, indicating that limitations affected their synthesis. These results suggest that the induced changes in *L. kaempferi* properties are partially up-regulated under N limitation, but that secondary compound synthesis was affected by external site-dependent factors other than N limited condition.

Effects of insect defoliation were studied on the formation of secondary cell walls of tracheids in *L. kaempferi* with a focus on the defoliation timing [65]. The secondary cell walls of tracheids produced in a defoliation year in *L. kaempferi* trees on which needles were attacked in July (Gypsy Moth, GM samples) or August (Larch sawfly, LS samples). GM samples produced non-lignified tracheids in the transition zone between earlywood and latewood, as well as thin-walled latewood

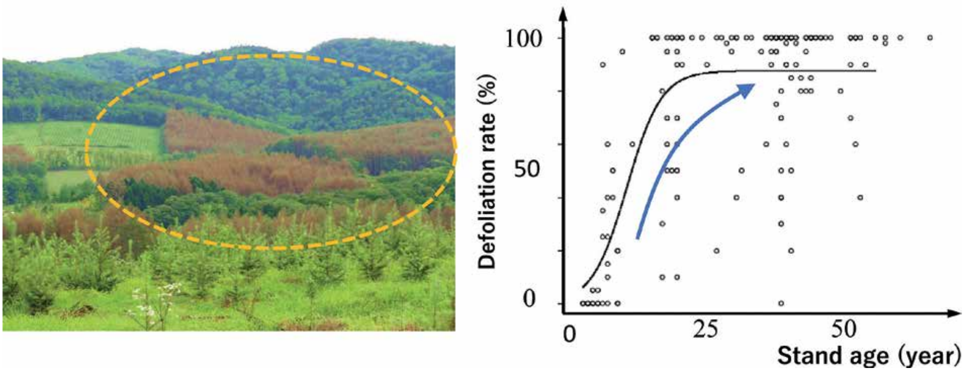

Figure 9.
*Yearly trend of larch sawfly in Hokkaido Island (after Fujita et al. [63]). Larch sawfly (Pristiphora erichsonii) grazes mainly shoot-shoot needles, and then defoliation increases with increasing stand age due to high proportion of short-shoot needles (Photo courtesy by: Kitami office of Hokkaido Pref.) defoliation rate was detected by aerial photography, and recently by unmanned aerial vehicle (UAV). After the grazed area was detected by these methods, defoliation rate (%) is expressed against plantation area by the records. The dashed orange circle shows larch plantations that have been damaged by larch sawfly grazing (left; photo courtesy by: Kitami office of Hokkaido Pref.). The right graphs indicate the relationship between the defoliation rate (5) and stand age. As larch sawfly (*P. erichsonii*) mainly grazes on the short-shoot needles, and the defoliation rate increases with increasing stand age as the proportion of short-shoot needles also increase with stand age. For* Larix kaempferi *seedlings, current year shoot and root growth were decreased with defoliating intensity and traumatic resin canals were also observed from stem cross-sections (after Fujita et al. [63]).*

tracheids, and non-lignified tracheids were observed near the cambial zone in LS samples following defoliation for two consecutive years. Changes in wood structure depend on the date of insect defoliation and that insect defoliation affects the formation of secondary cell walls of tracheids, presumably in response to inadequate photosynthates supply due to defoliation. We can recognize them as "white ring" as reported in birch and poplar [66, 67].

5. Genetics and breeding effort

5.1 Russia and China

Collection of larch seed, representing larch over the whole range of genetic and geographic variation has been discussed between Russian and Swedish authorities since the 1950s [14, 22]. The objective of the Russian-Scandinavian Larch Project is to study the genetics of the four main larch species within Russia, *L. sukaczewii* Dyl., *L. sibirica* Ledeb., *Larix gmelinii* Rupr., and *L. cajanderi* Mayr., and to make future research on genotype-environment interaction in other parts of the northern hemisphere possible [14, 22]. Up to the end of 2000 seed and wood cores were collected from 1005 larch trees distributed over 16 regions and 45 stands. In addition to that larch seed has been bulk collected from eight stands. Collected seed from 802 open-pollinated families were tested for seed germination in the summer of 2000 [22]. The average germination rate of the seeds was 25%, but with great variation among larch species [22, 39].

5.2 Japan

5.2.1 Brief history

Japanese forestry engineers successfully created hybrid larch F_1 with high tolerance to various stress and also improved timber quality. Efforts are also made to establish larch plantations with considerations to biodiversity management, however, the outbreak of diseases of Japanese larch are reported in even UK [68]. Elite tree of larch was selected 270 clones from 20 to 40 years old plantations during 1955–1961; these clones were originated from central Japan. Among them we preferred to use Dahurian larch originated from the Kuril Islands but not from Sakhalin Island because leaf senescence is delayed in the Kuril one [18, 69].

Seed orchards were made by randomly planted with elite trees of Dahurian arch and Japanese larch, and provided seeds of hybrid larch F_1 [14]. At 2000, we used hybrid larch F_1 for plantation of 300 hm^{-2} $year^{-1}$ (=800,000 planting stocks), however, these production activities were far from the demand of forestry industries [18]. This may be attributed to the low capacity of producing fertility of seeds of F_1. The crossing ratio fluctuated mainly depending on the pollen father of Japanese larch; it reaches 56.3% in the good harvesting year while it was 23.2% in the bad harvest year, based on DNA marker [70]. On the way of selection of ideal F_1, we isolated “Clean larch” (nick name of this new species) which showed a high growth rate and density in the stem (≒0.55); more than 20% larger than those of Japanese larch [17, 71].

5.2.2 Hybrid larch F_1

In northern Japan, hybrid larch F_1 (*Larix gmelinii* var. *japonica* × *L. kaempferi*; hereafter F_1) was produced to improve tolerance to grazing damage by voles and stem straightness, thus enhancing growth rate, timber quality [15, 70]. Nowadays propagation methods have improved, that is, cutting from only current seedlings, so the F_1 is becoming a principal afforestation tree species in northern Japan [14]. These new benefits bring a new plantation method from the traditional method: high planting density with several thinning to low planting density [18]. As shown in **Figure 10**, the relationship between the growth of the annual ring and bulk density ($kg\ m^{-3}$) in several trees [72]. From this, it is shown that the larch species keeps its high bulk density-independent of annual ring growth (over 320 $kg\ m^{-3}$ should be needed) because of clear change from “spring wood” to thick “summer wood.”

If we would plant larch with low density (standard planting density is 3000 ha^{-1}), we can keep commercial important wood strength. This means we can save our labor power in weeding in the initial stage of planting. Regional Forestry Institute proposes low-density plantation from 1,500 to 1,000 hm^{-2} [18]. With low density, we can expect the invasion of several kinds of species that regenerate at open gaps. As efforts are also being made to establish plantations with considerations made to biodiversity management, the invasions of these gap species may be beneficial. In addition, topics on diseases of Japanese larch in the UK should be considered [68].

5.2.3 Improve CO_2 fixation capacity of a forest ecosystem

Clear-cut harvesting is one of the mainly performed forest management methods but is it considered to be the cause of large CO_2 emissions. Understanding how this form of harvesting or logging affects site-specific CO_2 balance is important for determining a considerate management method, however, data on how timber harvesting affects the CO_2 balance of the ecosystem is still limited (**Figure 11**).

An experimental clear-cutting and plantation establishment study have been conducted in a cool-temperate mixed forest in northern Japan [73]. Before planting a the promising F_1 (*Larix gmelinii* var. *japonica* × *Larix kaempferi*), dwarf-bamboo: *Sasa* sp. was stripped to secure space. We obtained a complete series of pre- and post-harvest data on the net ecosystem CO_2 exchange (NEE) between the ecosystem

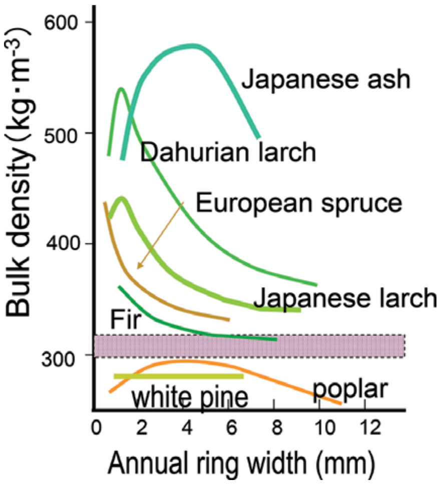

Figure 10.
Annual ring width and bulk density in several tree species planted in Hokkaido Island, Japan (Adopted from: Miyajima [72]).

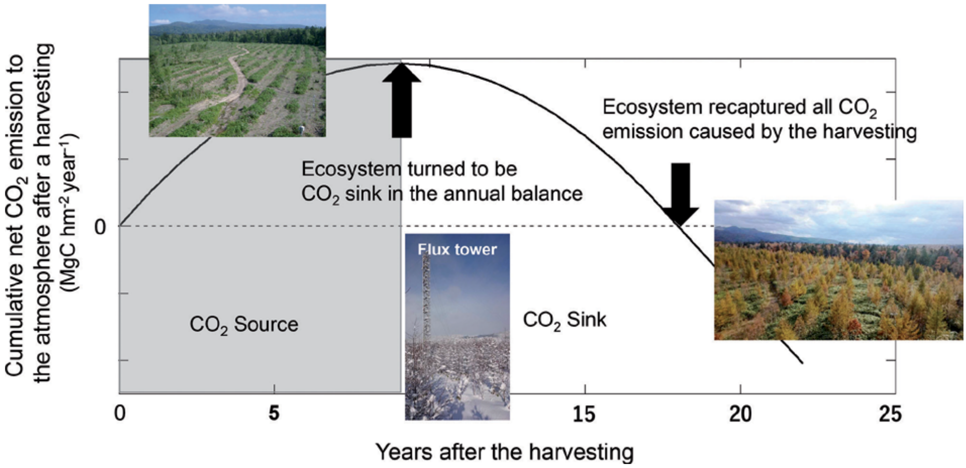

Figure 11.
Concept of the forest CO_2 balance after the harvesting from a spare mixed stand to make new plantation of hybrid larch F_1 (a view in mid-October in yellow color) at Teshio Experiment Forest located at northern most Japan (Takagi et al. [73]).

and the atmosphere until the disturbed ecosystem was once more a net CO_2 sink in the annual budget and recapture all the emitted CO_2 after the harvest and weeding. An over-harvested mixed forest, which had been a weak CO_2 sink with dense *Sasa* sp. (=dwarf bamboo) community was disturbed by the harvest of remaining trees and was replaced with a hybrid larch F_1 plantation. The ecosystem turned to be a large CO_2 emission source just after the harvesting in 2003, and the cumulative net CO_2 emission reached up to 15.4 MgC hm^{-2} at 7 years after the harvesting, then the “new” ecosystem turned to be a CO_2 retrieve mode (i.e., CO_2 sink in the annual budget). This ecosystem with F_1 recaptured all CO_2 emissions, 18 years after the harvesting in 2020, not considering off-site carbon storage in forest products. This means that a single harvest procedure works to change the CO_2 balance because the large invisible and long-lasting effect on the forest ecosystem CO_2 balance at the northern most experiment forest in Japan.

6. Ecophysiological responses of F_1 to environmental changes

We focus on the effects of environments (light, water) on larch species in Far East Russia to understand further responses of larch to the rapid change of environment including pollutants.

6.1 Russia and China

One of the topics will be described in this section to understand functional traits of larch in permafrost habitats (limited precipitation but rich in water via permafrost) in a continental climate, for example, Siberia (Russia): needle CO_2 assimilation, respiration, and intra-tree carbon transfer using ^{13}C labeling of mature larch trees. In China, the ecophysiological study is very limited but most studies were oriented CO_2 flux monitoring to contribute CO_2 balance in the atmosphere [74] but the acute estimation of non-photosynthetic organs [75] and soil respiration under different land-use [56]. Here we mainly focus on the ecophysiology of central Siberia studies.

6.1.1 Needle CO_2 exchange at Tura forest (Masyagina O. et al.)

The study area locates in the larch ecosystem (*Larix gmelinii* Rupr. Rupr.), which is a typical forest of the northern part of Central Siberia (Tura, Krasnoyarsk region, Russian Federation) with continuous permafrost presented (**Figure 12**). The climatic conditions of the study region and detailed characteristics of the chosen area are described by [77]. The study site (116 m^2) is a dwarf shrub-*Carex*-green feathermoss larch stand with an understory of *Salix* spp. The stand average age is 104 years as of 2013. The stand density is 9,052 hm^{-2}, the average tree height is 4.89 m, and DBH is 4.44 cm. Soil type is Typic Aquorthels.

6.1.2 Whole-tree ^{13}C-labeling experiments

At Tura site, nine mature larch trees were ^{13}C-labeled using whole-tree chambers (**Figure 12C** and **D**) in 2013–2014 (three trees in August 2013 and six trees in June and July 2014) [77]. A transparent plastic chamber (film thickness = 125 μm) was specifically designed to label the whole crown of the mature larch trees (about 104-year-old as of 2013). The chamber size (about 7 m^3) was related to the dimensions of the target trees. Mixing fans were used to enable uniform distribution of ^{13}C-labeled CO_2 inside the chamber and for regulation of the inner air temperature and humidity, to prevent the photosynthetic apparatus from damaging due to the high temperature, which is expected in the closed chamber under intensive insolation [78, 79]. The ^{13}C-labeling procedure is described by Masyagina et al. [77].

During the growing seasons (05 August 2013–19 September 2013 and 14 May 2014–15 September 2014), we sampled larch organs and tissues (brachiblasts: short-shoot needles; auxyblasts: long-shoot needle), twigs, phloem, xylem, and roots) for isotopic analysis of bulk $\delta^{13}C$ from ^{13}C-labeled trees on the selected dates (−1 = before labeling), 0 (-1 = before labeling, 0 = day of the labeling, 1, 4, 8, 15, 28, 40, 60, 75, and 90 days after labeling). In the following years after labeling, the sampling was undertaken monthly from June to September (2013–2018). Needles have been collected from the sun-exposed position of the larch crown between 11:00 and 18:00 hours (sampling was not conducted on rainy days). After collection, the needles were inactivated with a microwave oven at the middle regime (ca. 350 Watt) for 3 minutes to stop enzymatic and metabolic activities [80] (in details, please refer to * part in **Figure 12**).

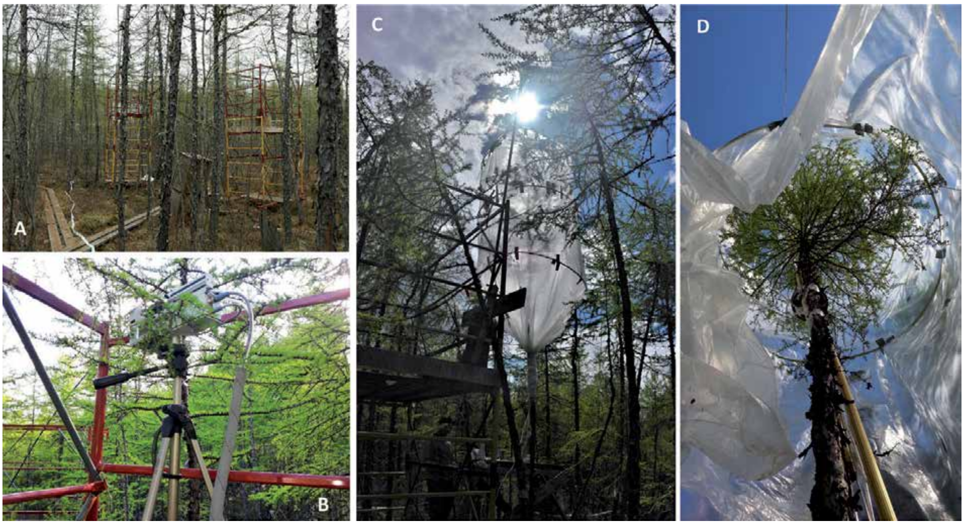

Figure 12.
*Tura site layout (A), needle CO_2-exchange measurement (B), whole-tree ^{13}C-labeling experiment in June 2014 (C) and ^{13}C-labeled mature larch tree inside the chamber (D) [76]. *: The samples were then dried for 48 hours at 60°C and ground to a fine powder. $\delta^{13}C$ analyses of the samples were done using automated device HeliView (MediChems Engineers Co., Ltd., Chungcheongnam-do, Korea) comprised of mass-spectrometer and gas chromatograph (N = 1447) in NRC Kurchatov Institute, Moscow, Russia), Isotope Ratio Mass Spectrometer Isoprime 100 (Isoprime), Elemental Analyzer Vario Isotope Cube (Elementar) (N = 118) in Sukachev Institute of Forest SB RAS, Federal Research Center "Krasnoyarsk Science Center SB RAS," Krasnoyarsk, Russia), and an elemental Analyzer-isotope ratio mass spectrometer (N = 395) in the Stable Isotope Laboratory of the Natural Resources Institute Finland (Luke) in Helsinki (Finland). All devices were inter-calibrated by analyzing the same samples to ensure the same accuracy of the devices. **: CO_2 gas exchange rates were measured at the middle part of the larch crown as it demonstrated mean values of pigment contents and average needle CO_2 gas exchange rates compared to the bottom and upper part of the crown. To access all control trees, we constructed 4-m-height monitoring tree towers. CO_2 exchange rates were calculated per needle projection area (μmol CO_2 m^{-2} s^{-1}). Foliar projection area was measured with flatbed scanner CanoScan LiDE 700F and further calculated using software «AreaS» 2.1 (developer Permyakov A.N., www.ssaa.ru).*

6.1.3 CO_2 exchange measurements in larch needles

Seasonal CO_2 exchange of larch needles of six non-^{13}C-labeled larch trees in the mid-June, mid-July, and mid-August of 2013–2014 was measured using an infrared gas analyzer Walz GFS-3000 equipped with the chamber for conifers (3010-V80) with the inner area of 8 cm^2 as described by Masyagina et al. [77] (**Figure 12B**). *In vivo* measured with the infra-red gas analyzer, the net CO_2 assimilation represents a net balance between the carbon flux entering the leaf (the gross photosynthesis) and departing the leaf simultaneously (the photorespiration and the mitochondrial respiration in the light) [81] (in details please read ** part in **Figure 12**).

6.1.4 CO_2 exchange of larch trees in permafrost habitats

Diurnal dynamics of needle CO_2 exchange of larch trees were studied over the growing season of 2013–2014. CO_2 exchange values varied seasonally from −3.6 to 8.9 μmol CO_2 m^{-2} s^{-1} in 2013 and −3.9 to 9.1 μmol CO_2 m^{-2} s^{-1} in 2014. Similar maximal values of photosynthetic rates for *Larix gmelinii* have been reported from Eastern Siberia (2.7–10.1 μmol CO_2 m^{-2} s^{-1} by Vygodskaya et al. [82] and ca. 11.3 μmol CO_2 m^{-2} s^{-1} by Korzukhin et al. [83]), Central Siberia (7.5–11 μmol CO_2 m^{-2} s^{-1} [50]), and China (8–11 μmol CO_2 m^{-2} s^{-1} [74]).

Midday depression of photosynthesis has been registered almost in all studied trees except for one individual in July of 2013. The most profound depression was found in June of both years when soil water accessibility remains little due to the shallow active soil layer (<20 cm) and in July 2014. In the permafrost zone, the physiological activity of *L. gmelinii* is essentially dependent on soil water supply from the seasonally thawed active layer [84].

Diurnal dynamics of photosynthesis slightly varied among months of the growing season, for example, its length per 24 hours varied in the range of 11–16 hours due to environmental conditions. For example, in June, photosynthesis was registered from 6 a.m. to 9 p.m. in 2013 or 7 p.m. in 2014; in July, photosynthesis was active from 5 a.m. to 8–9 p.m.; in August, photosynthesis lasted from 6 a.m. to 5 p.m. in 2013 and from 8 a.m. to 7 p.m. in 2014. Interesting, the average values of photosynthesis were on a similar level of ca. 1–3 μmol CO_2 m^{-2} s^{-1} (**Figure 13**) in various months. In mid-July, we observed a slightly higher rate of CO_2 assimilation compared to the rest of the growing season.

6.1.5 Intra-tree δ^{13}C carbon transfer (^{13}C labeling of mature larch trees)

To understand how C is traveling and allocating within a larch tree, we conducted several ^{13}C labeling experiments at the beginning (June), in the middle (July), and at the end (August) of growing seasons of 2013 and 2014. Here, we will discuss only June-labeled trees, namely labeled on June 10–12, 2014. The main C-accepting tree organs were needles and long shoots; their enriched δ^{13}C values achieved about 1700‰ in several hours after the ^{13}C-labeling experiment completion (**Figure 14**). Our study showed similar CO_2 assimilation capacity that resulted in the insignificant variation in ^{13}C excess (about 136 ± 1% [mean value ± SE], CV = 4%, unpublished) in needles among the trees labeled in various periods of the growing season (mid-June, mid-July, and mid-August) in the day of the ^{13}C-labeling experiment. It is a very interesting phenomenon since we found high variation in the environmental variables [77]. The ^{13}C-enrichment of phloem, xylem, twigs, and roots did not exceed 500‰ just after the labeling experiment (**Figure 14**).

In the year of the ^{13}C-labeling experiment, the highest decay rate of δ^{13}C was observed in needles and long shoots (**Figure 14**, panel 0). Two months after the ^{13}C-labeling experiment there were peaked δ^{13}C values (about 150‰) found in phloem due to intensive transfer of C at that time. At the end of the growing period of 2014 (year of a ^{13}C-labeling experiment), the average δ^{13}C values in yellow needles and long shoots were ca. 300‰, in twigs and wood (phloem and xylem),

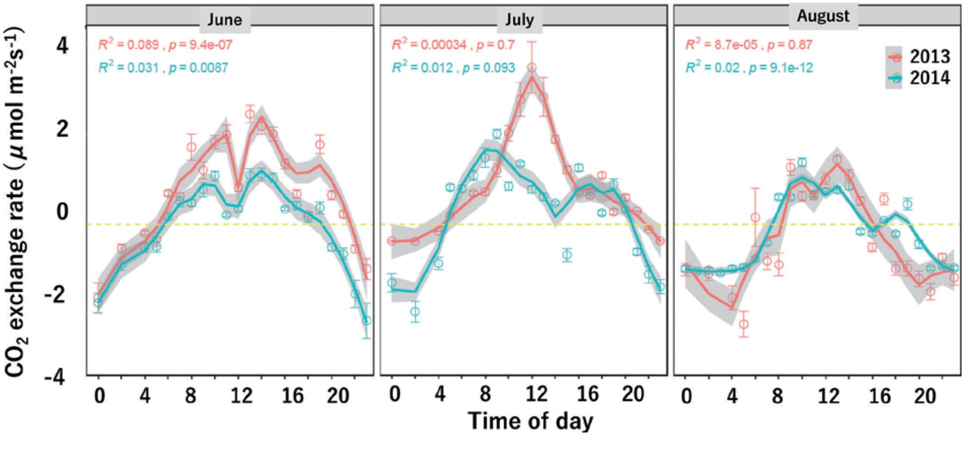

Figure 13.
Differences in diurnal curves of needle CO_2-exchange rate for different months of the growing season (June–August of 2013 and 2014) in permafrost habitats. Trends are loess regressions. Grey shadows represent confidence intervals (standard error) of the regression.

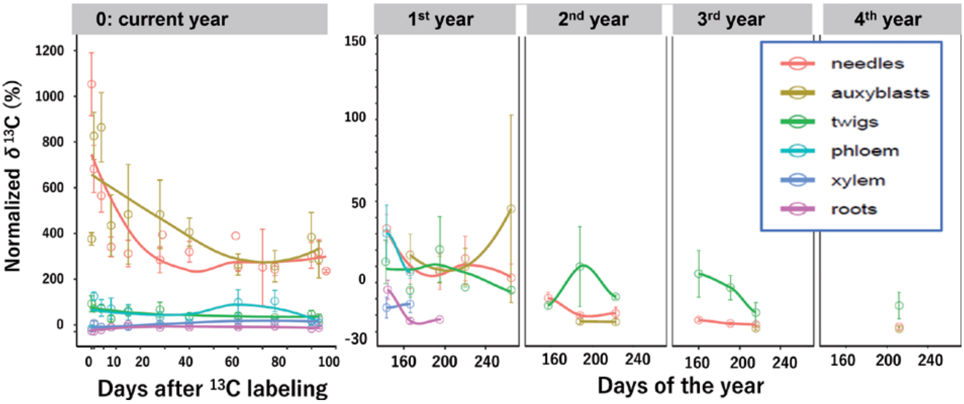

Figure 14.

*Dynamics of normalized δ^{13}C (mean ± SE) over 2014–2018 in various organs and tissues (short-shoot needles: brachiblasts, long-shoot needles: auxyblasts), twigs, phloem, xylem, and roots) of larch (*Larix gmelinii *Rupr. Rupr.) trees, which were ^{13}C-labeled in June of 2014. Grey panel represents year after ^{13}C-labeling: 0—a year when ^{13}C pulse-labeling was conducted; 1–4—year are following the 0 year. Trends are loess regressions; 0 day at 0 year is a day when ^{13}C labeling was conducted. DOY, day of the year.*

were ca. 21‰, and in roots were about −14‰. Such a high build-in C amount in senesced larch needles plays an important role in the metabolism of soil microbiota, including mycorrhiza, since it is an easy-destructive substrate.

Enriched δ^{13}C values have been observed in studied tissues of trees at least over 4 years after ^{13}C-labeling experiments (**Figure 14**, panel 1–4). At the beginning of the following growing season on 23 May, 2015, we registered enriched δ^{13}C values (from −22 to 49‰) in all larch organs and tissues. The most ^{13}C-enriched organs were needles, long-shoot needles, phloem, and twigs. It pointed to the intensive usage of the last-year C reserves in the early spring (bud-break period) for growth processes that confirms our previous results [77]. In other words, carbon is being involved in the exchange processes within a tree for a long time. However, Kagawa et al. [85] showed that after 2–3 years, there was little ^{13}C excess left in the needles of larch saplings. These differences from our results may be due to the age differences since Kagawa et al. [85] ^{13}C-labeled saplings of larch of heights of 10–73 cm.

6.1.6 Response of larch species to environmental changes in China (Y. N. Wang)

At present, Dahurian larch (*L. gmelinii*) showed a continuous distribution in the northeast of Inner Mongolia and the northwest of Heilongjiang Province, and the distribution in the north and central part of Heilongjiang Province with forest fires [46, 47]. Since *L. gmelinii* distributed in China is located in the southern margin of the global northern forest, there is no climatic suitable area for *L. gmelinii*, and all the climatic indicators cannot reach the optimum level for growth and development.

Forest dynamics of larch in NE China is strongly regulated by forest fires [46, 57]. Stand density in the young and middle-stage (around 100 years) is relatively high (about 2,300 hm^{-2}), but it sharply decreased over 100 years after the fire, and reached about 1,500 hm^{-2}. The aboveground was estimated to be around 115 Mg ha^{-1}. There was an altitudinal gradient of above biomass at Daxingan Mt. range (latitude 47 N to 52 N from 85 to 42 Mg hm^{-2}, respectively), and 32 Mg hm^{-2} at Tura in Siberia (N62) [46]. Ecosystem productivity of China to Siberia decreases sharply with increasing latitude (**Figure 15b**) accompanied by an increase in shoot/root ratio [55].

Under the government of China's environmental program known as Returning Farmland To Forests (RFTF = NFCP), about 28 million hectares of farmland have been converted to tree plantations. This has led to a large accumulation of biomass carbon, but less is known about underground carbon-related processes [56]. One permanent plot (25 years of observation) and four chronosequence plot series comprising 159 plots of larch (*Larix gmelinii*) plantations in northeastern China were studied. Both methods found significant soil organic carbon (SOC) accumulation (96.4 gC m^{-2} $year^{-1}$) and bulk density decrease (5.7 mg cm^{-3} $year^{-1}$) in the surface soil layer (0–20 cm), but no consistent changes in deeper layers, indicating that larch planting under the RFTF program can increase SOC storage and improve the physical properties of surface soil. Nitrogen depletion (4.1–4.3 gm^{-2} $year^{-1}$), soil acidification (0.007–0.022 pH units $year^{-1}$), and carbon/nitrogen (C/N) ratio increase (0.16–0.46 per year) were observed in lessive soil, whereas no significant changes were found in typical dark-brown forest soil.

This SOC accumulation rate (96.4 gm^{-2} $year^{-1}$) can take 39% of the total carbon sink capacity [net ecosystem exchange (NEE)] of larch forests in this region and the total soil carbon sequestration could be 87 Tg carbon within 20 years of plantation by approximating all larch plantations in northeastern China (4.5 M hm^{-2}), showing the importance of soil carbon accumulation in the ecosystem carbon balance. By comparison with the rates of these processes in agricultural use, the RFTF program of reversing land use for agriculture will rehabilitate SOC, soil fertility, and bulk density slowly (69% of the depletion rate in agricultural use), so that a much longer duration is needed to rehabilitate the underground function of soil via the RFTF program. Global forest plantations on abandoned farmland or function to protecting farmland are of steady growth and our findings may be important for understanding their underground carbon processes.

However, climate change has significantly affected the geographical distribution, population pattern and community productivity of *L. gmelinii* in recent years. Prediction of potential distributions under future climates shows its geographical distribution range gradually reduced, and may even move out of the north altogether, future climate warming will have a negative impact on the distribution of *L. gmelinii* in China.

6.1.7 Japan

In northern Japan, Japanese larch (*Larix kaempferi* Carr.) has been planted widely in reforestation schemes. This larch was introduced to northern Japan from the central subalpine region of Japan in the 1870s [15, 18]. Larch species were believed to be the most promising tree species for afforestation, similar to the Sakhalin fir (*Abies sachalinensis* Masters) native to Hokkaido, because they grow

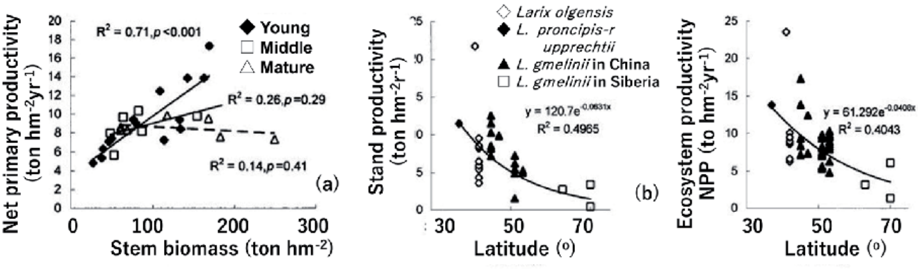

Figure 15.
Biomass of stem and net primary production (NPP) (a), latitudinal gradation and strand productivity or ecosystem productivity (b) (Modified from: Wang et al. [55], with the authors' permission).

more rapidly and are more tolerant against cold than other traditional silvicultural species in Japan [8, 13]. Consequently, larch covers widely Hokkaido Island, matching the shape of the island [13], however, there were some problems with Japanese larch on Hokkaido because Japanese larch is introduced species. It was susceptible to diseases such as root rot and shoot blight, and to grazing by redback voles [14]. Growth traits are mainly analyzed by plant biomass productivities, and improvements have been made on survival and timber quality [13, 39].

7. Environmental factors affecting larch species

Izuta [86] well summarized the current condition of the impact of environmental pollution on forest and farmland ecosystem. Since the 1960s, with the rapid economic development, air pollutants have impacted forest health and vigor in NE Asia [87]. SOx pollutants were reduced by desulfurization equipment during the 1970s; however, NOx including precures of O_3 has hardly decreased because it is mainly produced by traffics [86]. Lockdowns applied amid the Covid-19 pandemic may decrease the rate to about 7 ppm year^{-1} between 2019 and 2020 as found in O_3 emission in Europe [88]. Finally, we also discuss ECM under environmental change.

7.1 CO_2

Effects of environmental changes on larch growth under elevated atmospheric CO_2 concentration [CO_2]. Globally, [CO_2] has been increasing steadily since the Industrial Revolution. As CO_2 is a resource for photosynthesis in green plants, an increase in [CO_2] appears to be favorable for photosynthesis and the growth of trees. Although net assimilation rate and growth of trees were enhanced by elevated [CO_2], the positive effects on light-saturated photosynthetic rate (P_{sat}) do not persist over the long term [87]. Trees usually acclimatize to elevated [CO_2] conditions. The P_{sat} of plant species grown at elevated [CO_2] decreases with time to the same level as that at ambient [CO_2], which was found by Tissue and Oechel [89]. This trend was especially observed under severe conditions, for example, infertile soil, root restriction, and/or dilution of nutrients in the plant body [87]. This phenomenon is called "down-regulation" or photosynthetic adjustment [87, 89].

We should also consider the combined effects of high [CO_2] and N deposition as a promoter of tree growth [90]. Physiological effects of nitrogen deposition on CO_2 fixation are summarized as follows: Eguchi et al. [91] studied the photosynthesis of 2-year-old Japanese larch seedlings raised under ambient [CO_2] (360 μmol mol^{-1}) and high [CO_2] (720 μmol mol^{-1}), using environmental control growth cabinets (Phytotron). They found that high [CO_2] increased the light and CO_2-saturated photosynthetic rate (P_{max}) of seedlings and changed the inner structure of needles of the seedlings grown in high-nutrient soil. The internal mesophyll surface area per unit needle surface area (A^{mes}/A or S^{mes}) increased with high [CO_2], leading to a reduction in diffusion resistance of CO_2 [91]. They concluded that the increase in the photosynthetic rate at high [CO_2] was mainly due to easier transport of the CO_2 to chloroplasts in needles.

Growth response and nutrient status of 2-year-old Japanese larch seedlings raised under different [CO_2] during two growing seasons were determined by using an open-top chamber (OTC) [92]. At the end of the second growing season, high [CO_2] increased the total biomass of Japanese larch seedlings, while only root biomass increased by elevated [CO_2] was detected at the end of the first growing season. The different [CO_2] levels did not give rise to any difference in nutrient concentration in the plant body, or in mycorrhizal formation in roots of seedlings.

The greater total biomass under high [CO_2] was due mainly to the increased root biomass during the first growing season, allowing better absorption of nutrients and stimulation of growth during the second growing season [92].

The xylem structure of Japanese larch seedlings under a combination of two [CO_2] and nutrient regimes in phytotron for one growing season [91, 93]. Stimulation of secondary growth by high [CO_2] was observed only with the high nutrient treatment. High [CO_2] also increased the stem base diameter and changed some anatomical features of the tracheids, especially cell diameter. Development of more branches was observed for *L. sibirica* seedlings grown under high [CO_2]. However, elevated [CO_2] had no effects on dry-matter production or tree height of the seedlings.

7.2 Ozone

Ozone (O_3) in the troposphere is recognized as a widespread phytotoxic air pollutant. Since even ambient levels of O_3 adversely affect growth and physiological functions, such as photosynthesis, of forest tree species, this gas is considered to be one of the most important factors involved in forest decline and reducing photosynthetic production in the USA, Europe, and Japan [10, 94]. The effects of oxidants on plants have been studied since the 1940s and have been reported that ozone generates reactive oxygen species such as O_2- and H_2O_2 in leaves, having adverse effects on fatty acids in protoplasm and proteins in leaf. Based on experimental studies, Japanese larch is relatively sensitive to O_3 exposure compared with other tree species in Japan [95]. In general, sensitivity to O_3 of plants is greatly affected by growth conditions, such as temperature, light intensity, and soil moisture and nutrient status.

Watanabe et al. [96] reported that the sensitivity to O_3 of Japanese larch seedlings grown in soil supplied with N at 50 kg N hm^{-2} $year^{-1}$ was less than at 0 and 20 kg N hm^{-2} $year^{-1}$. Nitrogen-induced changes in sensitivity to O_3 must therefore be considered in risk assessment of O_3 toward Japanese larch. Since [O_3] in Hokkaido is currently low, the negative effect of O_3 on larch species in this area may not be serious at present. However, relatively high [O_3], sufficient to induce a reduction in the growth of larch species, is estimated in other parts of Japan [95]. Furthermore, [O_3] has been increasing in Japan over the last two decades [10]. This trend will continue with an increase in precursors of O_3, such as nitrogen oxides and volatile organic compounds, especially in the East Asian region [10].

It is predicted that photosynthetic production in terrestrial plants of the northern hemisphere will be reduced by more than 20–30% due to O_3 in the near future [94]. The effect of high [O_3] on larch species should therefore be considered because larch is dominant species in northern hemisphere.

According to the recent NO_2 trend in Asia [97], we should pay attention to the rapid increase in NO_2 emission and also the Biological volatile organic compound (BVOC) of larch as a precursor of O_3 [98].

Based on the statistics of EU (LRTAP: Long-range Transboundary Air Pollution) and US (EPA: Environmental Protection Agency), the NO_2 emission from Asia reached about 43 Tg NO_2 $year^{-1}$ which is four times larger than that from EU or UAS (**Figure 16**). Therefore, we should know this evidence and try to give O_3 tolerance to larch plantation as suggested by Watanabe et al. [96].

The biomass of Japanese larch decreased at 80 ppb, but it was lower at low O_3 (<5 ppb) compared to 25 ppb [100]. This phenomenon is regarded as hormesis [101]. As almost all practical production of larch seedlings is done in the suburbs, we tried to use ethylenediurea (EDU) to moderate the adverse effects of elevated O_3 on larch seedlings [102]. An effective concentration of EDU is 400 mg EDU

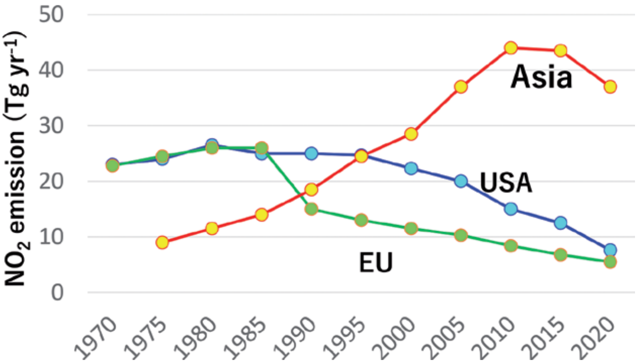

Figure 16.

Yearly trend of NO_2 emission of three regions. Data are cited from Akimoto [99] and Kurokawa and Ohara [97]. 1990~: EU-LRTAP convention; USA-EPA Air Pollutant Emission and NIES (adopted from Qu et al. [5]).

L^{-1} applied as soil drench it protects both Japanese and F_1 plants against toxicities induced by exposure to elevated O_3 for up to 3–4 years. Methods using container grown seedlings in forestry practices are including mushroom production.

7.3 N deposition

Nitrogen is often a limiting resource for plant growth in the forest ecosystem [82], and N fertilization frequently results in increased photosynthesis and enhanced growth of trees. Excessive amounts of N can nevertheless have a negative effect on the physiology and growth of the forest ecosystem. Forest declining due to high N load is suggested to have occurred in some coniferous forests, as recognized by the N saturation story [103]. N deposition has been increasing dramatically, especially in East Asia [11]. The main sources of atmospheric N deposition are anthropogenic emissions due mainly to fossil-fuel burning, and food production, relating mainly to agricultural waste and overuse of N-fertilizer. In Hokkaido, the annual deposition of N (NH_4 + NO_3) has increased to about 1.2 kg N hm^{-2} $year^{-1}$ (as of 2012 [104]). Unfortunately, this increment will continue in the near future.

However, negative effects of N loading have not been observed yet. For example, N loading N load did not significantly affect the growth and net photosynthetic rate of Japanese larch seedlings grown in the soil of Andisol [96]. Furthermore, no growth and photosynthetic stimulation of Japanese larch and hybrid larch F_1, grown in a mixture of clay loam, peat moss, and vermiculite with balanced fertilizer containing N and other nutrients such as P and K [4]. In contrast, growth and photosynthesis of Japanese larch seedlings grown in a mixture of clay loam and well-weathered pumice (nick name Kanuma), were stimulated by balanced fertilizer [92, 93]. Although the experimental periods differed among these studies, the soil type used in the experiment may be one of the important factors that induce the difference in the response of larch to N load (or fertilization).

Enzymes in N metabolism are affected by irradiance conditions [105], so that growth and photosynthetic responses of larch to N load are also regulated by light levels. Qu [4] examined the effects of different light intensities (8, 16, 32, and 100% of open condition) and two fertilization regimes (high/low) on seedlings of Japanese larch and F_1 raised in a mixture of clay loam, peat moss, and vermiculite (the fertilizer was composed of balanced nutrients, like Hyponex: N:P:K = 6:10:5 and micro-elements). When light intensity exceeded 16%, dry-matter growth of Japanese larch was greater than that of F_1, independent of fertilization regimes.

However, the growth of Japanese larch in high-nutrient conditions was dramatically suppressed at 8% light intensity. This result indicates that high N load will make Japanese larch susceptible to shading, which was also found in nursery condition [106]. Ryu et al. [15] examined the effects of high N load on growth and ectomycorrhiza infection of Japanese larch, Dahurian larch, and their hybrid F_1 seedlings growing in serpentine soil at low light intensity (8% against open) assuming forest floor conditions. It is well known that ECM symbiosis is important for the growth of host plants by assisting in the uptake of water and essential nutrients and by excluding heavy metals [34, 107]. No significant effects of N load on growth and infection by ECM were found. Inadequate light intensity and shortage of essential materials are suggested as possible factors for this phenomenon.

Several researchers indicated a fertilization effect of N load on other tree species such as Sugi-cedar (*Cryptomeria japonica*), Siebold's beech (*Fagus crenata*), and deciduous oak (*Quercus serrata*), however, N load did not necessarily stimulate the growth of larch species [4, 15, 108]. On the other hand, N load to the level of 50 kg N hm^{-2} $year^{-1}$ will not negatively affect the growth of larch species. We may have to avoid shading seedlings when we introduce multistoried forest and/or natural regeneration to Japanese larch forest under high N load [4, 106], because of N-load induced reduction in shade tolerance. Although N is an essential nutrient for plant growth, information on its combined effects with other environmental factors on larch species in northern Japan is very limited [15] and further investigation is needed.

7.4 Soil acidification

In northeast Asia, pine and larch forests have declined in the vicinity of industrial or urban regions. Important factors causing this decline are the decrease in available nutrition and the increased metallic toxicity induced by soil acidification [86, 109]. Likely factors limiting the growth of plants in acid soil are the high acidity itself, phytotoxic metals such as aluminum (Al) or manganese (Mn), and reduced availability of important elements for plant growth [86]. However, infection with ECM fungi improves tolerance to environmental stresses by reducing the toxicity of metals [110]. The ECM role in growth responses of larch species under acid soil is therefore important in clarifying the effect of soil acidification on larches. The growth response of Japanese larch seedlings infected with several ectomycorrhizal fungi and raised under different soil acidification levels (proton concentrations of 10, 30, 60, and 90 mmol H^+ kg^{-1}) [109]. They quantified the ECM symbiosis that leads to improvement of the rhizosphere of larch seedlings. The results suggested that water-soluble phytotoxic elements (such as Al^{3+} and Mn^{2+}) and essential elements (such as Ca^{2+}, Mg^{2+}, and K^+) in soil increased with increasing soil acidification.

Concentrations of Al in the root and Mn in needles also increased. It is well known that Al^{3+} reduces the growth of roots, and Mn^{2+} replaces Mg^{2+} bound to the carboxylation enzyme (Rubisco; ribulose-1,5-bisphosphate carboxylase/oxygenase) and reduces photosynthetic activity [27]. Photosynthesis and the total dry mass of larch seedlings infected with ECM fungi were higher than in controls in all soil treatments. Also, the total dry mass of ECM seedlings was less at an acid level of 90 mmol H^+ kg^{-1} than in unacidified ECM control seedlings; the ratio was about the same as without ECM infection.

As shown in Choi [109], severe soil acidification reduces the growth and photosynthesis of Japanese larch. At a lower level of acidification, ectomycorrhiza will help the larch to maintain growth, but will not help at severe acidification level. Based on the growth response to the concentration ratio of base cation (Ca^{2+}, Mg^{2+},

K^+) to Al^{3+} (i.e., BC/Al ratio; BC/(Al + Mn) ratio) in the soil solution or water extract of soil, the sensitivity of Japanese larch to soil acidification is similar to that of Sugi-cedar, Red-pine and Sieblod's beech [86, 109]. Soil acidification is important in the long term as, if the deposition rate of acid exceeds the rate of recovery of buffering capacity by weathering, the acid neutralization capacity of soil will be reduced in the future and soil pH will decrease. Considering that the lifespan of the tree is long, a serious reduction of growth of larch species due to soil acidification could occur in the future.

7.5 Role of ECM

Since the 1950s, with the rapid economic development, air pollutants (NOx, SOx, Ozone: O_3) and increasing CO_2 have impacted forest health and vigor. The photosynthetic rate is usually reduced by elevated CO_2 under root restricting conditions. SOx pollutants were reduced by desulfurization equipment during the 1970s; however, NOx has hardly changed because it is mainly produced by diesel cars [86]. NO_2 is converted by O_3 and NO via UV radiation [111]. In addition, atmospheric CO_2 concentration [CO_2] has increased since the Industrial Revolution and has reached around 418 ppm at the current rate of 2.2 ppm $year^{-1}$. However, lockdowns applied amid the Covid-19 pandemic decreased the rate to about 7 ppm $year^{-1}$ between 2019 and 2020.

Except for O_3 (troposphere or ground-level O_3), sufficient CO_2 and adequate N are regarded as the productive atmospheric environment for forest trees. We summarize the effects of changing environment (CO_2, N deposition, and O_3) on the growth of larch and larch-ECM interactions.

7.5.1 Responses to elevated CO_2

In many cases, we found down-regulation of photosynthesis under elevated CO_2, even in a FACE (Free Air CO_2 Environment [87]) system. We expected ECM to act as a carbon sink and moderate down-regulation in photosynthesis, although for red pine seedlings inoculated with a kind of ECM (*Pisolithus arhizus*) down-regulation was not observed [109].

The same trend was expected in larch. Hybrid larch F_1 was planted in the FACE for 3 years and tended to fall down because of increased above-ground biomass [110]. After 5 years of CO_2 fumigation in FACE, Japanese larch decreased biomass allocation to branches and increased it by about 20% in the stem compared with ambient CO_2. In contrast, birch (*Betula platyphylla* var. *japonica*) and kalopanax (*Kalopanax septemlobus*) allocate about 10% less biomass to their stems. Almost no anatomical structure changed with elevated CO_2 [112].

7.5.2 Responses to elevated O_3

Ozone levels have been increasing around the northern hemisphere in the past several decades [94]. With the GIS method, Watanabe et al. [113] predicted that the growth of Japanese larch (*Larix kaempferi*) would be reduced by elevated O_3 around the Kanto plain, and in contrast, the decline will not be as significant in northern Japan. Ozone concentration is generally high in the suburbs due to the oxidation of NO_2 (exhaust gas: NO from diesel cars plus UV) [111]. This cycling of NOx under UV can lead to the generation of O_3 ($NO + O_3 \rightleftharpoons NO_2$ + UV-radiation) in suburban green areas around big cities. Based on screening using OTCs (open top chambers), Yamaguchi et al. [95] summarized the O_3 sensitivity of potted 18 tree seedlings in Japan, and among them, the Japanese larch showed a moderate sensitivity. What about the hybrid larch F_1 under elevated O_3?

The specific difference in O_3 sensitivity was examined between Japanese and hybrid larch F_1 seedlings planted on the ground of OTCs (<5, 25, 45, and 80 ppb). The growth of both larches was significantly suppressed by 80 ppb (**Figure 17**; [14, 114]). The biomass of F_1 seedlings decreased under 25 ppb, compared to <5 ppb, but this was due to its heterosis and maintained a similar biomass with Japanese larch seedlings in elevated O_3 treatments.

7.5.3 Elevated CO_2 and O_3

Plants usually close their stomata under elevated CO_2 to reduce the absorption of O_3. We examined the effects of elevated O_3 (80 ppb) on the growth and ECM infection and diversity of hybrid larch F_1 seedings under elevated CO_2 in OTCs [37]. Under elevated O_3, ECM infection rate and species diversity were reduced; however, these trends were moderated by elevated CO_2 (600 ppm). Only early successional types of ECMs were found at ambient and elevated CO_2. However, larch specialist *Suillus* sp. was dominant under elevated O_3 (**Figure 18**).

This evidence suggests that a kind of ECM, *Suillus* sp. may support the growth of the host plant, larch hybrid F_1. As Qu et al. [32] suggested the photosynthetic rate in larch species infected with multiple ECM species was higher compared to when infected with a single ECM species. This phenomenon is recognized as follows: most ECM activity depends on soil pH; some ECMs prefer low pH but some require neutral or high pH (6 ~ 8), such as *Rhizopogon rubescens* [115]. Aluminum (Al) is released below pH < 4.5 and inhibits root growth. These are species-specific traits, and this may be the reason why multiple ECM infections may benefit the host plants more significantly.

7.5.4 Nitrogen deposition and elevated O_3

The combined effects of N and elevated O_3 were studied in seedlings of two broadly distributed species: Siebold's beech and larch, with the use of OTCs. The beech is classified as highly sensitive to O_3 [95]. With increasing N (NH_4NO_3), O_3 sensitivity of the beech increased in terms of Accumulated Exposure Over Threshold (AOT) of 40 ppb O_3 (AOT40). In contrast, O_3 sensitivity of Japanese larch decreased with increasing N up to 50 kg N hm^{-2} $year^{-1}$. However, hybrid larch F_1 had slightly increased O_3 sensitivity with 50 kg N hm^{-2} $year^{-1}$ under free-air O_3 exposure [116], which may be due to decreased leaf life-span with N application [100].

In general, phosphorous (P) is the second most important nutrient after N; for the growth of hybrid larch F_1, an adequate supply of P and N is required. Mg was

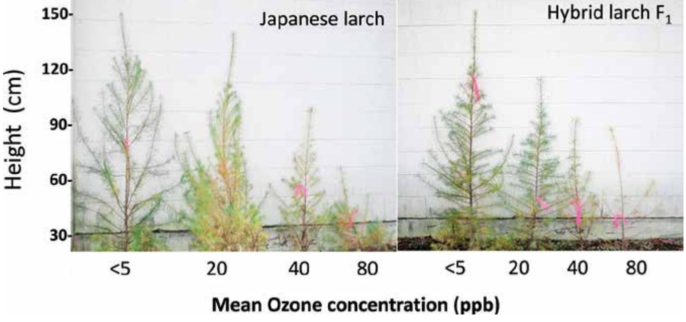

Figure 17.
Ozone concentrations and height in Japanese larch and its hybrid larch F_1 (Adopted from: Kita et al. [14], with authors' permission).

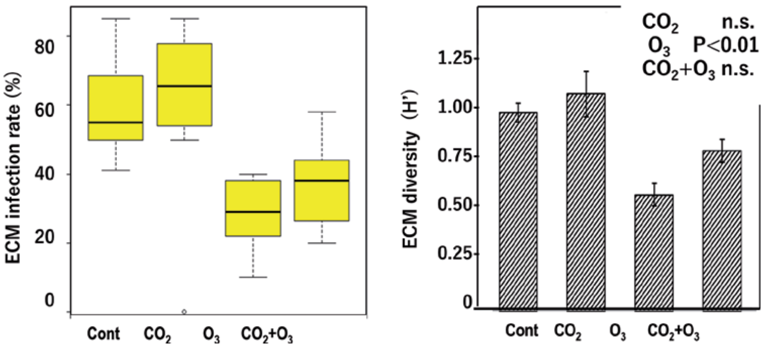

Figure 18.
Infection rate and diversity of ECM of hybrid larch treated with combination of elevated CO_2 *(600 ppm) and* O_3 *(80ppb) (Adopted from: Wang et al. [37]).*

the limiting element in the nursery of Hokkaido University [117]. In this edaphic condition, we examined the effects of N deposition (NH_4NO_3) on the growth of hybrid larch F_1 for 8 years. Surprisingly, as a result, except for N application to F_1 by the second year, almost no difference in the growth of F_1 was found between the N treatment and the control (=no N application). Based on DNA analysis of the ITS region in symbiotic ECM, most of them infecting Japanese, Dahurian, and hybrid larch F_1 were nitrogenous species [38], and were not altered by N application.

7.5.5 ECM-larch: Conclusion

We expect a new CO_2 sink when planting a new plantation in northern Japan. In Far East Russia and central Siberia [4, 5], they recognize the real essential role of the larch ecosystem on permafrost area, and they try to increase their timber quality to use genetically ideal larch and conserve permafrost ecosystem. Japanese larch is intensively used after considerable improvements in timber utilization. After harvest, we should make plantations with container-grown seedlings to save labor and attain high plantation efficiency. If we make new plantations with hybrid larch F_1, we should ensure larch plantations do not increase N deposition under elevated O_3. To make planting stock of F_1, we should inoculate larch seedlings with ECMs (*Suillus* sp.) for increased tolerance against environmental stress.

8. Conclusion

In conclusion of this chapter, it can be said that urgent considerations should be made to moderate elevated ground-level O_3 including dynamics of NO_2 as precures of O_3 against green infrastructure around big cities [5, 102], as larch forests is a vital component of global as well as local resources.

In this chapter, emphasis was made on the essential role of the larch ecosystem for environment conservation via highly forest management techniques. For this objective, we should point out detailed aspects of the larch forest ecosystem, specially developed on permafrost in Far East Russia and NE China. Recently, TV programs suggest the fear of melting of permafrost under changing environment in Alaska even though biological importance has been revealed back in the 1990s [23]. With the melting permafrost layer, many kinds of greenhouse gasses (CO_2, CH_4, N_2O, NO_2 as precures of O_3, etc.) may be released and destroy our environment. Further knowledge on the ecophysiology of larch is still needed [50, 118–120], phylogeny [121], as the wise use of larch ecosystems will contribute to nature conservation and the sustainable use of the world's natural resources.

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Author details

Laiye Qu[1*], Yannan Wang[2], Oxana Masyagina[3], Satoshi Kitaoka[4], Saki Fujita[4,5], Kazuhito Kita[6], Anatoly Prokushkin[3] and Takayoshi Koike[1,4*]

1 Research Center for Eco-Environmental Sciences, CAS; University of Chinese Academy of Sciences, Beijing, China

2 Jiangxi Academy of Forestry, Nanchang, China

3 Sukachev Institute of Forest, Federal Research Center "Krasnoyarsk Science Center SB RAS", Krasnoyarsk, Russian Federation

4 School of Agriculture, Hokkaido University, Sapporo, Japan

5 Forestry and Forest Products Research Institute, Tsukuba, Japan

6 Forestry Research Institute, HRO, Bibai, Hokkaido, Japan

*Address all correspondence to: lyqu@rcees.ac.cn and tkoike@for.agr.hokudai.ac.jp

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Chapter 6

Pinus patula Plantations in Africa: An Overview of Its Silvicultural Traits and Use under SDG

Wubalem Tadesse and Teresa Fidalgo Fonseca

Abstract

Pinus patula Schiede ex Schltdl. & Cham. is a forest tree species native to Mexico, widely cultivated in tropical and subtropical regions of the world. In Africa, the plantation of the species has gained considerable interest being represented in different African countries, and probably being the most widely planted pine in tropical Africa. The species traits and the diversity of wood use to highlight the importance and usefulness of the species in the tropical regions of the African continent. The aim of this chapter is to review the state-of-the-art on the knowledge of the species, namely their characteristics and their growth dynamics, present information on productions, silvicultural management and biotic vulnerabilities, and summarize the effects on biodiversity and relevance on carbon stock. Knowledge of species biological and silvicultural traits supports decision-making on sustainable forest management and contributes to the achievement of the Sustainable Development Goals of UN Agenda 2030.

Keywords: Patula pine, species traits, silviculture, forest sustainability, afforestation

1. Introduction

About 1/3 of the world's total land surface is forest (4.06 billion hectares). In terms of major regions, the forest area in America represents 40% of the total (21% for South America and 19% for North and Central America), Europe contributes 25%, followed by Africa, with 16% of the world's forested area. Asia has 15% and Oceania has the remaining 5% [1]. The importance of forests is widely accepted and is addressed in the UN 2030 Agenda for Sustainable Development, being broadened and considered in Sustainable Development Goal (SDG) 15—Life on Land [2]. The agenda is a commitment of the United Nations to eradicate poverty and achieve sustainable development by 2030 worldwide, ensuring that no one is left behind. Forestry can help to achieve SDGs, especially SDG 15 which specifically considers to protect, restore, and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, halt and reverse land degradation and halt biodiversity loss [2], but also SDG 1 (income to fight poverty) or SDG13 (carbon capture and storage) among others. See Baumgartner [3], for a comprehensive discussion on how sustainable forest management could contribute to achieving other Sustainable Development Goals.

Global Forest Resources Assessment 2020 report [1] informs that the world has lost a net area of 178 million ha of forest since 1990, with the highest annual rate of net forest loss in 2010–2020 occurring in Africa (net forest loss of 3.9 million ha). The same source [1] reports that the rate of net forest loss has increased in Africa in each of the three decades since 1990. Forest resources have paramount socioeconomic and ecological importance in many African countries. They contribute to poverty alleviation in a variety of ways and provide important support mainly for sub-Saharan Africa's economic, social, cultural, and environmental development, especially in rural areas. Over two-thirds of all Africans rely directly or indirectly on forest resources for their livelihoods. However, deforestation and forest degradation are hampering the forest resources of many countries in the continent. These forests have come under severe pressure from a growing demand for forest products, particularly fuelwood, and the expansion of agricultural land. The use of planted forests is a strategy that supports and benefits the achievement of SDG 15. To this end, knowledge of the biological traits and silviculture of forest species and decision on what appropriate species to use is essential.

With this review, the authors aimed to gather information on one of the most widely used species in plantations in Africa, *Pinus patula*. The literature on the species is considerable but is rather limited when restricted to Africa. The information here presented intends to provide guidance on the use of this species as a management option to consider when the aim is to ensure the sustainability of forest resources on this continent.

2. Distribution and ecology of *P. patula*

P. patula Schiede ex Schltdl. & Cham., commonly known in English as Mexican weeping pine or patula pine, is a native species from Mexico [4, 5]. The species is largely used for plantations in tropical and subtropical regions of the world, including South America, Central and Southern Africa, Indonesia, Australia, and New Zealand [6 and references herein]. The area planted with *P. patula* worldwide is approximately 1 million hectares, of which 95% correspond to plantations in Central, Eastern, and southern Africa [6]. It is widely planted in different African countries. Probably is the most widely planted pine in tropical Africa. **Figures 1** and **2** present a *P. patula* plantation in Ethiopia. The species is of particular importance in Kenya, where it accounts for about 25% of all forest plantations. It is also important species in Madagascar, Malawi, South Africa, Tanzania, and Zimbabwe. For instance, in South Africa, 54% of the total forested area is pine forest, with *P. patula* being the most widespread species, covering an area of about 375,000 ha [6].

P. patula is a species that tolerates most soils and will grow in grassland. The species thrives best when there is good water supply but can also overcome unfavorable conditions [7]. It is often found in pure dense stands, but the occurrence is discontinuous, and now, for much of its range, it develops only in areas inaccessible to agriculture. Over its entire distribution range, *P. patula* can be found associated with *Platocthispa gregorii* and *P. teocote*, with which it is said to have hybridized. Other mixtures mentioned include association with *P. montizumae* and *P. rudis*, *Abies religiosa*, *Taxus mexicana*, and hardwood species of such genera as *Acer*, *Cercis*, *Fagus*, *Tilia,* and *Liquidambar* [7]. *P. patula* demands deep, well-drained soils and grows best in the mist belt regions of South Africa above 1 000 m elevation. In southern Africa, the species are generally planted in areas with mean annual temperatures below 17°C [8]. In Ethiopia, the species develops best with good water supplies but can also survive adverse conditions in 1900–3000 m [9].

Figure 1.
Pinus patula *plantation picture in south West Ethiopia (photo credit: Reinhold G).*

Figure 2.
Pinus patula *plantation picture in Western Ethiopia (photo credit: Wubalem Tadesse).*

In southern Africa female flowering starts when trees are 2–3 years old, and male flowering 1–2 years later. The flowering of both male and female cones occurs in August–October, with usually a secondary flush of only female cones in January–May. In Kenya, two flushes of male and female flowering normally occur in April–May and October–November, coinciding with the rainy seasons. However, there are also records of female flowering happening throughout the year. Studies in Zimbabwe showed that synchronization of pollen shedding and female receptivity was good at 1500 m altitude, whereas pollen shedding occurred progressively later at lower altitudes. Outcrossing is predominant, and pollination is mainly by wind. Female cones mature in 22–30 months. The production of viable seeds starts when trees are 5 years old, and is prolific in 8–10-year-old trees. Seed dispersal is usually by wind, but sometimes also by birds, rodents, or people [5].

P. patula is a very demanding species in terms of light. It is considered an aggressive pioneer species that grows easily in forest gaps created by fire. In countries, such as South Africa, Swaziland, and Zimbabwe, it is now considered a serious weed, forest edges, moist grasslands, and road cuts [5].

3. Silvicultural characteristics

3.1 Tree species characteristics, growth dynamics, and yield

P. patula grows very fast. Under favorable conditions, it may attain a height (h) of 15 m after 8 years and 35 m after 30 years [5]. The species can reach a diameter at breast height (d) of up to 1.2 m [7]. The bole is usually straight and cylindrical (see **Figures 1** and **2**). Sometimes the trunk fork produces two or more stems. When the growing area of the tree is large (wide-spaced plantation), the crown tends to spread out. In terms of shape, the crown can be rounded or spiral-shaped. The bark has distinct characteristics depending on the tree's stage of development. When young, it has a reddish-orange color and is scaly. In contrast, the mature bark is gray-brown and vertically ridged [7].

Mean annual increments in volume (MAI) are 10–40 $m^3ha^{-1}y^{-1}$, in southern Africa ranging from 18 to 28 $m^3ha^{-1}y^{-1}$. In East Africa, the species can show higher yields than in southern Africa due to a shorter dry season. The total yield (including thinning removals) under appropriate conditions maybe 630–700 m^3ha^{-1} [5]. In South Africa, when planted on sites with well-drained soils, mean annual temperatures below 17°C and at altitudes above 1000 m, the species has an expected MAI ranging from 10.9 $m^3ha^{-1}y^{-1}$ to 27.2 $m^3ha^{-1}\,y^{-1}$, good stem form, and wood properties [10].

According to the Wood Data Base [11], this species presents a wood density of around 450 kgm^{-3} (dried basis) and 580 kgm^{-3} (12% of moisture). Wood density may vary depending on the altitude, presenting higher values when plantation occurs at lower altitudes, instead of higher ones. The length of rotation also influences wood density. In Tanzania, density at 12% moisture content was reported to increase from 380 kgm^{-3} for 12-year-old trees to 510 kgm^{-3} for 30-year-old trees [5]. *P. patula* is mainly used for firewood, timber (boxes, general purpose), posts (treated with wood preservative), pulpwood, shade, and ornamental [7]. The wood is highly appreciated for glued laminated timber for carpentry and furniture after the knots have been removed. It is also suitable for hardboard, particleboard, and wood wool. *P. patula* is an important source of pulpwood [5].

3.2 Silvicultural guidelines

The species is used in forestation and afforestation as a high-forest managed system, with an even-aged stand structure (see **Figures 1** and **2**). Although mixtures can be found (see Section 2), typically, *P. patula* is managed in pure stands.

Initial spacing for *P. patula* in most countries is from about 2.4 m to 2.75 m or 3 m, corresponding to individual growing areas around 6–9 m^2. Generally, for sawlogs, closer spacing is recommended for knot-free wood. Wider spacing is recommended on poorer sites. Sawlog regimes in common use are conceived to have about 250 $treesha^{-1}$ with an average diameter at breast height of 45 cm at a 45-year rotation. For pulp schedules, rotations range from 15 years in Swaziland to 25 years, as recommended in South Africa [7].

During the first year after planting 2–3 operations to control spontaneous vegetation are required, to reduce the occurrence and growth of competitive vegetation and optimize yield. Ndlovu et al. [10] referring to the species grown on pulpwood regime, point out the importance of vegetation management impacts, vegetation management, by keeping the stands free of vegetation, showing a positive significant influence on diameter at breast height, basal area, and volume growth, with implications on rotation end stand volume. The vegetation management is performed through manual removal or chemically with the application of glyphosate.

The response of *P. patula* to fertilizers is site-specific, with some research studies pointing to the beneficial effect of phosphorus and potassium fertilizer in stand growth [12, 13] and in correcting the growth decline observed between first and second rotations [14]. Results by [15] support the beneficial effects of phosphorus but not nitrogen on the early growth of the species. The application of N significantly depressed the diameter growth of the trees.

P. patula is a species with limited self-pruning, justifying the use of artificial pruning, to reduce the risk of fire and improve access within the stand ("low pruning"). In general, trees are pruned at 4–6 years of age to a height of 2.5 m. In pulpwood plantations, no further pruning is done, although pruning to a height of 6 m has been recommended to reduce fire risk. For the production of sawn timber, both dead and live branches up to a height of 7(−12) m ("high pruning") are removed to produce knot-free wood [5]. In a thinning trial, in Malawi, Missanjo et al. [16] studied the effect of first thinning and pruning on height, diameter at breast height, and volume growth in individual trees. The authors concluded about the importance of both practices in *P. patula* plantations, recommending their use to maximize the increase in volume production. The highest diameter and volume growth were registered when thinning and pruning were applied, while the highest growth in height was observed where there was pruning and no thinning.

Intensive silviculture aiming to maximize productivity and gains is traditionally carried out in commercial timber plantations within South Africa during the re-establishment phase, however, there is limited information as to the rotation end benefits of this input [10]. Thinning characteristics depend on initial spacing, site quality, and end product and affect tree growth through its effects on growing space. In Zimbabwe and South Africa, in forestry systems aimed at producing sawlogs, the ultimate goal is to have a stand of more than 400 treesha^{-1} of about 45 cm diameter, implying 25–35-year rotations. In Zimbabwe plantations with an initial 3 m × 3 m spacing (density of 1100 treesha^{-1}) may be thinned twice, with density reduction to 650 treesha^{-1} after 6–8 years, and to 400 treesha^{-1} after 12–15 years. In Madagascar, heavy thinning is recommended to reach a target density of 200–250 treesha^{-1} when the trees are 15 years old. For pulpwood schedules, rotations of 15–25 years are normal, resulting in trees with a bole diameter of about 30 cm [5].

The mechanization of harvesting has been transforming the practice of thinning, leading to increased use of row thinning to gain access to a stand with machinery while restricting the application of selective thinning in the areas between machine trails. Ackerman et al. [17] advert that this procedure carries the risk of irregular stand structure with resulting adverse effects on crown growth and its eccentricity and plasticity, potentially negatively affecting saw timber quality and volume production from the stands at final felling. These consequences, confirmed by the authors on a plantation of *P. patula* in South Africa, assume particular importance when the species is managed in long rotations, aiming for the production of sawlogs.

A brief characterization of silvicultural guidelines for the management of *P. patula* is depicted in **Table 1**. The prescriptions of the silvicultural activities that are carried out are in accordance to the references mentioned for plantations in Africa.

3.3 Models to support decision-making processes

To the best knowledge of the authors most of the biometric studies involving the development of tree and stand-level models for *P. patula* report to the species in Mexico (e.g. [6, 18]), but there are records on equations developed with data collected in other countries. In the GlobAllomeTree international web platform ([19], see also [20]), 14 out of the 38 allometric equations to estimate tree volume (v)

Year	Activity	Description	Observation
0	Site preparation		
0	Stand establishment: artificial regeneration (plantation)	Initial spacing about 2.4 m to 2.75 m, or wider (e.g. 3 m).	Closer spacing is recommended for saw log schedules. Wider spacing is recommended on poorer sites
1	Control of spontaneous vegetation	2–3 weeding operations to reduce the occurrence and growth of competitive vegetation	The vegetation management is performed either manually or chemically
4–6	Pruning	Trees are pruned to a height up to 2.5 m	The operation is recommended both for pulp and sawlog schedules. For the production of sawn timber, further pruning interventions are recommended at a later age to a height of 7 to 12 m. Whenever pruning coincides with thinning, it is carried out on the trees to be retained.
6–15	Thinning	One to two thinnings. Reduction of stems per hectare gradually or in a single heavy thinning, to a final density of 200 to 400 treesha^{-1}	Thinning applies for sawlog schedules but is not usual for pulp schedules. Frequently combines mechanical row thinning along with selective thinning within the strips with trees.
15–45	Final harvest	Final cut of the living trees.	Rotation of 15 to 25 years for pulp projects, rotation of 25–45 years for saw log schedules

Table 1.
Silvicultural guidelines for the management of pure P. patula *plantations.*

or biomass (b) of *P. patula* were developed with data collected in the African countries of Tanzania (6), South Africa (5), Kenya (2), and Rwanda (1). A summary of the available models with the specification of the explanatory variables and response variables is provided in **Table 2**, along with the reference studies ([19], references herein). The equations to estimate tree volume or tree biomass used as regressors one or a combination of the following variables, diameter at breast height (d), tree height (h), crown height (ch), tree basal area (g), and age (t). Listed in **Table 2** is a stand-level-type model allowing to estimate stand volume (V) using as input data age, stand basal area (G), and dominant height (h_{dom}).

Regarding growth models, data-driven models and process-based models are described for the species in East and South Africa [28–31]. Reference [32] presents and discusses progress made on predictive models for growth and wood quality for the species managed in South Africa for saw timber. Delgado-Matas and T Pukkala [33] elaborate on growth models for the species in Angola. Complementary information from a literature review of both growth models and tree volume equations for species in Africa can be found in this reference [33].

In the context of decision support models, two additional specific contributions should be highlighted, these are references [34] and [35]. The study by Gadow and Kotze [34], based on *P. patula* spacing experiments, refers to tree survival and maximum tree density. The authors estimated the self-thinning line (Reineke [36]) for the species and found it to be positively related to site index, with higher maximum density values in the better-quality sites. Mugasha et al. [37] provide a comprehensive study on the development of individual and stand models to support the decision on optimal rotation length for the species in Tanzania.

Allometric equation	**Response variable**	**Explanatory variables**	**Geographical location**	**Reference**
40551	Tree volume	d , h	Kenya	[21]
47709	Tree volume	d	Rwanda	[22]
41516, 41586	Tree biomass	d , ch	South Africa	[23]
45212, 46720	Tree biomass	d , h	South Africa	[23]
45871	Tree biomass	d	South Africa	[23]
38145	Tree volume	g	Tanzania	[24]
38656	Tree volume	d , h	Tanzania	[24]
39372	Tree volume	g , h , t	Tanzania	[25]
44165	Tree volume	d , h	Tanzania	[26]
46201	Stand volume	G , t , h_{dom}	Tanzania	[27]

Table 2.
Existing models for Pinus patula *developed for African forest systems (from [19]).*

3.4 Pests and diseases

Most of the insect pests harming *P. patula* are defoliators, particularly of the order Lepidoptera, the most noted being the families Arctiidae, Lasiocampidae, Noctuidae, and Saturniidae. The damage starts from the nursery stage by means of cutworms, several leaf rollers, and defoliators. The pests in plantations include adult leaf-eating beetles, adult bark beetles (mottled pine bark weevil) as well as sucking insects, such as pine wooly aphid. Diseases of the species comprise foliage leaf cast, tip die-back of the branches, and armillaria root rot [7]. *Sphaeropsis sapinea* was also mentioned as an economically important pathogen of *P. patula* in South Africa, causing rapid die-back and mortality of hail-damaged trees [37]. *Fusarium circinatum*, known as the pitch canker fungus (PCF), is one of the most important pathogens to natural and industrial pine forests, being a serious threat to *P. patula* [38]. Post-establishment mortality with *P. patula* in commercial plantations is currently the most pressing operational concern and poses a threat to the continued use of pure *P. patula* as a species [8]. *P. patula* has been identified as being particularly susceptible to the PCF [39]. Long-term control strategies include the usage of alternative species, hybridization, breeding, and selection programs to improve resistance [38]. Knowledge regarding the molecular basis of pine *Fustiaria circinatum* host-pathogen interactions could assist efforts to produce more resistant planting stock. Visser et al. [40] identified molecular responses underlying resistance against *F. circinatum*. According to the results found, delayed response and impaired phytohormone signaling contribute to pathogen susceptibility in *P. patula*.

In addition to insect pests and diseases, rodent-induced damage has also been reported in first-rotation pine plantations in South Africa 4–5 years after planting. Tree mortality was attributed to changes in the structure of shrub and grass assemblages within the plantation, causing rodents to feed on the pine trees [41].

3.5 Effects on biodiversity and relevance on aboveground carbon stock

The usage of *P. patula* in a plantation system does not appear to negatively impact biodiversity. In fact, in a study conducted by [42] in western Kenya, the authors reported there was no significant variation in woody species richness among disturbed primary forest, old-growth secondary forest, middle-aged secondary forest, mixed indigenous plantation, and *P. patula* monoculture plantations. Old-growth and middle-aged secondary forests had relatively higher woody species diversity indices than *P. patula* monoculture plantations, but the difference was not statistically significant. Regarding the contribution to aboveground carbon offset, results indicated that *P. patula*, had a lower carbon stock than other types of forests. This was explained by the smaller stem diameter and wood specific gravity lower than or comparable to the other species and forest types in the study, being predominantly associated with the schedules adopted for the plantations of *P. patula* of short (pulpwood) to medium rotation length. A short rotation length affects biomass accumulation (which will be comparably lower) and wood density (also lower, as noted in Section 3.1).

4. Interest of *P. patula* within the framework of the sustainable development goal (SDG) 15

Major change processes in forests around the globe are deforestation, afforestation, and reforestation (**Figure 3**). Deforestation and Afforestation represent the transfers between forest and other land use classes. Reforestation occurs when forests regrow after temporarily having had below 10% canopy cover, but were considered forests throughout that time [43]. Reforestation envisages the recovery of forest areas and can be achieved with the plantation of forest species. Forest plantation is expanding in African countries. The great majority of planted trees are exotic species chosen for their capability to grow rapidly to produce wood of desired quality. *Eucalyptus* is the most widely planted genus covering 22.4% of all planted area, followed by *Pinus* (20.5%).

Well-managed forest plantations can help to alleviate pressure on natural forest resources. Further, as depicted in **Figure 3**, both processes of afforestation and reforestation act in the opposite direction of degradation of (forest) land. Two SDG targets of SDG 15 are explicitly identified [2]—ensuring the conservation, restoration, and sustainable use of terrestrial and inland freshwater ecosystems and their services, particularly forests (15.1); promoting the implementation of sustainable management of all types of forests, halting deforestation, restoring degraded forests, and increasing afforestation and reforestation globally (15.2).

The use of forest species, such as *P. patula*, considered in this review, in forestation or afforestation programs in Africa can be beneficial in counteracting or minimizing the pattern of forest area loss reported in [1]. To achieve better results, further research should be carried out on the species. The review summarized in the previous sections shows that there are gaps in knowledge for the species outside its natural range. Specific issues to prioritize in research embrace optimal silvicultural guidelines and mortality at young stages of development under an adaptive forest management perspective. Intensive silviculture is carried out within commercial

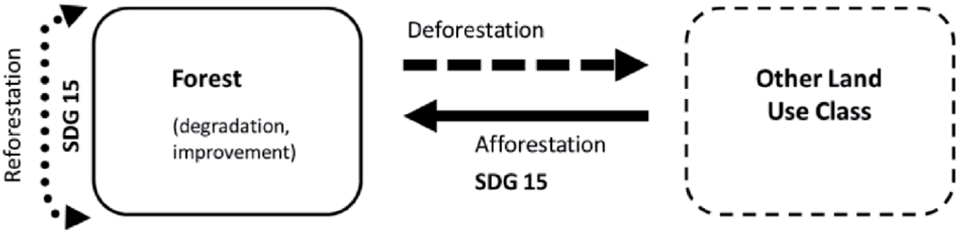

Figure 3.
Major forest changes processes with a reference to SDG 15. Based on [43].

timber plantations within South Africa during the re-establishment phase, however, there is limited information as to the rotation end benefits of this input. Post-establishment mortality with *P. patula* in commercial plantations is currently the most pressing operational concern and poses a threat to the continued use of pure *P. patula*. Research on these topics is, therefore, encouraged.

5. Conclusions

P. patula is a very fast-growing tree and the most widely planted pines species within African commercial plantations. The species is planted in Ethiopia, Kenya, Tanzania, Malawi, Zimbabwe, Madagascar, and South Africa and is mainly used for timber and firewood. The increase in forest area and its maintenance in good conditions contribute to the Sustainable Development Goals of UN Agenda 2030. Besides the positive impacts explicitly associated with SDG 15, positive effects of the forest sector might be expected in SDG 1 (income to fight poverty), SDG 6 (freshwater), and SDG 13 (carbon capture). Achievement of any of the goals requires appropriate knowledge of the forest species traits and their adequate forest management. The review on the species here presented aims to help accomplish these purposes, by providing information on the species and identifying research priorities.

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Conflict of interest

The authors declare no conflict of interest.

Author details

Wubalem Tadesse[1,2*] and Teresa Fidalgo Fonseca[1,3,4]

1 IUFRO Division 1 - Silviculture, Unit Ecology and Silviculture of Pine, Austria

2 Ethiopian Environment and Forest Research Institute (EEFRI), Addis Ababa, Ethiopia

3 ECAV – CIFAP, Department of Forestry Sciences and Landscape Architecture University of Trás-os-Montes e Alto Douro, Vila Real, Portugal

4 Forest Research Centre (CEF), School of Agriculture, University of Lisbon, Tapada da Ajuda, Lisboa, Portugal

*Address all correspondence to: wubalem16@gmail.com

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Chapter 7

Management of *Pinus pinaster* Aiton for Wood and Resin Production: A Technical-Financial Feasibility Analysis

André Sandim, Maria Emília Silva, Ana Cristina Gonçalves, Margarida Tomé and Teresa Fidalgo Fonseca

Abstract

Maritime Pine sector is an important agent for promoting economy and sustainable development in Portugal. Among the products explored in these forests are wood and resin. The objective of this work was to evaluate the technical and financial viability from the creation of three simulated exploration scenarios: Wood Exploration (W), Resin (R) and Wood + Resin (WR), for this it was defined a loss of 16% of wood volume in resined forest stands and created operational models that defined the costs, investments and revenues that made it possible to prepare cash flow for each scenario and apply the Internal Rate of Return (IRR) feasibility analysis indicators, Net Current Value (VAL), Net Profitability Index (NPI) and Discounted Pay Back (DPB). Scenario R presented the best indicators, however, with absolute values that were not attractive and that the W and WR scenario presented viability but with a DPB at 10 years of age. The loss of wood was not compensated by resin production. The indicators of the WR scenario fell short of those observed in scenario R. Increased market price of resin and scale gain can overcome wood loss and ensure added value in the joint exploration of wood and resin.

Keywords: internal rate return, net current value, net profitability index, discounted pay back, forestry, scenarios

1. Introduction

Maritime pine (*Pinus pinaster* Aiton) is a large, rustic pioneer species that can reach up to 25 m in height and 60 cm in diameter at 60–70 years [1], is undemanding about the soil, but tolerates little soils of heavy texture and soggy [2]. Studies indicate that there were two subspecies of maritime pine in Portuguese territory. The subspecies *P. pinaster atlantica* EH del Vilar occurs along the coast from the North Alentejo and Extremadura, extending north through the coastal dune systems, while the subspecies *P. pinaster escarena* (RISSO) K. Richter has a Mediterranean continental occurrence [3].

The maritime pine wood has multiple applications. In general, the use of wood is associated with the characteristics of the tree, notably the diameter of the logs.

Larger-diameter logs (above 35 cm) are used for unrolling or producing sheets for application in furniture, interior decoration, and carpentry. Logs with an intermediate diameter (between 20 and 35 cm) are, as a rule, used for sawing for construction and furniture, while logs that are between 14 and 20 cm are used for sawing for the production of boards for boxing, pallets, among others. The wood of smaller diameter (less than 14 cm) has its most notable use for milling for the pulp and energy. In addition to wood, the bark of the trees is used for the production of substrate for use in agriculture and the resin for the chemical industry [2].

Every approach that is taken to maritime pine stands, in order to justify the investment in resources and efforts in the study of this species, it must be preceded by a recovery of its benefits and applications in the economy and its impact and importance on the society in which it operates. Data from the Portuguese National Forest Inventory (IFN6) shows that maritime pine is the third most common species in terms of land area, with 713,000 ha [4].

The large representation of maritime pine in Portugal translates into relevant economic results for Portuguese society. The report 'The Pine Row in 2019' [5] informs those maritime pinewoods constitute the largest carbon reservoir in the national forest in Portugal and generate 57,843 jobs in 8516 companies that enabled the generation of 1225 million euros in Gross Added Value and a turnover of 4384 million euros with an export value of 1876 million euros. Despite the strong numbers, the maritime pine forest area [4] and the productivity of the stands have decreased in the last decades [5] and thus justify the relevance of the approach of technical and scientific players in the production and dissemination of knowledge that allows the sustainable perpetuation of this important row.

Although the *P. pinaster* wood sector is well established in Portugal, like any product that falls under the laws of the free market, it has its own dynamics motivated by the quantities offered and demanded. It is important for the sustainability of the chain and its continuity, that there is a favorable balance of the income generated in relation to the expenses or production costs. In a generic analysis, it is possible to glimpse this relationship from indicator systems such as the Simplified System of Forest Product Quotations [6] of the Institute for the Conservation of Nature and Forests (ICNF), which presents the market prices of the cubic meter with bark of wood of various species, including maritime pine in public areas managed by ICNF. In the same value quotation line, the costs of timber harvest operations can be found in the Monitoring Committee of DGADR's forestry operations—Directorate General for Agriculture and Rural Development [7].

According to the aforementioned platforms, the average prices practiced by the maritime pine wood market for final cut (standing wood) in Portugal in 2020 was 34.56 euros for uncertified wood and 39.55(a) euros for FSC-certified wood [6]. As for the exploration costs, considering the mechanized process in the cut-to-length system where the felling and processing are done by the harvester equipment and the forwarder is fed back, the average cost indicated is 15.45(b) euros, and for transport the cost average to consider a distance of less than 30 km is 11.50(c) euros [7]. Ultimately, the final cost of the wood delivered to the consumer under the conditions presented should be close to 66.50(a + b + c) euros for uncertified round wood, plus the profits along the chain, capital costs, fees and taxes for the final cost.

In addition to wood, resin is another product that is explored in this species and adds important value to maritime pine economy. The application of the resin, as a product, is vast, but it can be summarized in two broad groups: turpentine, from which products such as pine oil, vitamins, dyes, flavorings and products for the cosmetic industry are made; and the rosin which is the basis for the production of glues, soaps, rubbers, lubricants, paints and various products for the chemical and pharmaceutical industry [8]. Beyond of economics benefits, the resin exploration has an important social gain, because its exploration induces the community nearby take care of the forest, what results in a more resilient forest since it is more guarded [9].

The values paid per kg of resin in Portugal are around 18 euros, which makes the activity attractive, and can result in gains of 50–500 euros per year per hectare [10]. Despite its attractiveness, it is important to reflect on the physiological aspect of the tree in resin production, as for resin exudation there is a physiological process stimulated by a wound that supposedly diverts resources to be used in the secondary growth of the plant [11] raising the question about a possible loss of wood productivity in tapped individuals.

Regarding the loss of wood biomass in forests submitted to resining was verified 20% reductions in the volume of *P. pinaster* in France [12]. In Portugal, there was a loss of 14% [13] in the diameter at breast height (d) and 16% in the total volume of trees [14] in *P. pinaster* stands. In India, decreases of 17.64% were verified in the diameter of *Pinus roxburghii* trees [15] while in *P. pinaster* stands in Spain, the diameter loss reached 33% [14]. In Germany, experiments conducted on *Pinus sylvestris* did not verify loss of dimensions of diameter, height or volume [16]. **Table 1** summarizes the references regarding the loss of dimensions of tapped trees.

It must be considered that although the resin exploration promotes some reduction in the production of biomass (wood), the economic results of the production of wood x resin might be viable. The viability of the business for *Pinus elliotti* in Brazil even with a loss of 9% of wood volume due to resin coating [18]. Regarding the financial performance of tapped *Pinus elliottii* stands in Brazil, in relation to non-resined and extraction costs and business profitability, it was found that extraction operations cost less than US$0.50 (0.42 euros[1]) per tree in forests that produce at least 2.5 kg of resin per tree per year, are profitable (have financial viability), which represented a result of 504.00 dollars (423.36 Euros[1]) per hectare of resinous forest and a gain of 17.76%, although there was a significant loss of wood volume for veneer and sawmill in the resinous forest [18].

References	**Country**	**Species**	**Age (years)**	**diameter (%)**	**Height (%)**	**Volume (%)**
Figueiredo apud Gomes [15]	Portugal	*Pinus pinaster*	—	—	—	16.0%
Figueiredo apud Maginni [15]	Italy	*P. pinaster*	—	8.0%	6.0%	—
Lemoine & Decourt [12]	France	*P. pinaster*	30	—	—	20.0%
Verma & Pant [17]	India	*Pinus roxburghii*	—	17.6%	—	—
Figueiredo [15]	Brazil	*Pinus elliottii*	23	6.0 a 14.9%	12%	14.9%
Figueiredo [18]	Brazil	*P. elliottii*	23	—	—	9.0%
Ferreira [13]	Portugal	*P. pinaster*	—	14.0%	—	—
Gènova et al. [14]	Spain	*P. pinaster*	93–109	33.0%	—	—
Van der Maaten et al. [16]	Germany	*Pinus Silvestrys*	115–140	0%	0%	0%

Table 1.
Selected articles for definition of volume loss with indication of country of origin, species, age and loss of dimensions (diameter, height and volume) due to the influence of resin.

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[1] Quotation on September 7, 2021.

Planning the harvest of forest stands is a complex task due to the large number of production factors and the interactions between these factors, which makes each question that must be answered by planning assuming characteristics of 'perverse problems' or 'Wicked Problems', a situation in which the dynamics of the interaction between the factors of production is so complex that the answers are multiple and changeable according to the scenario presented at the time [19].

In order to mitigate the difficulties in the elaboration of scenarios imposed by the 'Wicked Problems', it is necessary to consider the analysis of a set of factors, which, when interacting, are responsible for the behavior of a variable that one wants to verify [19].

In a forest, factors such as climate, relief, soil type, species genetics, degree of anthropization, form and intensity of exploitation affect the potential productivity of wood and resin, as well as their interactions, hence the objective overview of this chapter will be to analyze among the possible production scenarios of a forest stand, for standard site conditions, which one offers the most sustainable results and the best balance between cost and benefit of integrated production of wood and resin, based not only on production potential, but also on the results of financial viability indicators based on investments and planned cash flow.

The objective of this work is to evaluate the technical and financial feasibility of different management goals in maritime pine stands considering managing the stands for wood and/or resin. The specific objectives of the chapter are addressed into two distinct stages. At first, a timber harvesting model will be defined in a maritime pine stand, using the Modispinaster simulator [20, 21]. Afterwards, it will be defined an average coefficient of loss of productivity of wood as a function of the resin extraction process, namely the relationship between dendrological variables (diameter, age, stand density) and wood production. In a second stage, after defining the integrated wood x resin productivity balance, based on market prices and considering the full commercialization of the products, the investments, operational costs and financial results will be verified based on the indicators of internal rate of return, net profitability index, net present value and payback time. The best exploitation scenario will be indicated according to the resin extraction intensity and always considering the cycles and interventions indicated by the previous phase simulated in Modispinaster. In this way, it is intended to answer the question: How do the financial viability indicators respond considering the joint exploration of wood and resin? Considering the exploitation of only wood, only resin and wood with resin, which scenario is more attractive?

2. Methodology

This study is based on scenario creation and simulations; therefore, there is no real physical area for data collection. The data listed here were obtained through interviews conducted in 2021 with companies operating in the forestry market, notably with the exploration of maritime pine and are based on records of these companies and on the common sense existing in the market. Some characteristics of the hypothetical simulated study area were predefined, such as forest size, terrain characteristics, fuel consumption and wages and charges and others, such as the type of equipment used and the exploration system were considered as adjustable variables for obtaining the lowest-cost scenarios.

2.1 Methodology for determining the wood productivity adjustment factor due to the impact of resin coating

The impact of resin on tree growth and, consequently, on wood productivity was assessed in scientific literature for *Pinus* genus in countries where it is natural or cultivated (**Table 1**).

Some references (see **Table 1**) did not indicate the impact on volume variation due to tapping, but rather on the stem diameter and eventually on height. To obtain a measure of impact on volume and therefore, to measure the volume loss as a function of diameter reduction, the volume model (Eq. 1) developed for the species *P. pinaster* [22] was used. The equation allows to estimate the total stem volume (v, m^3) of a tree based on the diameter measured over bark at 1.30 m height above the soil level (d, cm). The equation was applied for each diameter class central value, starting at 10 cm, and then the process was repeated for the diameter class centre until 65 cm, with the reduction in percentage pointed out by the references in **Table 1** [13–15].

$$v = -0.073243082 + 0.005815280 \times e^{d^{0.458656754}} \quad (1)$$

The comparison of the volumes obtained for each operation was used as a proxy of the loss of volume.

In this way, with the variation of the inserted diameters, the volume variation was achieved. The density of green (wet) wood over bark of *P. pinaster* with an average age of 33 years is close to 0.8 $g.cm^{-1}$ [23], therefore considering that the stand of this study has an average age of 40 years, and that with increasing age, there is also an increase in wood density [24], it was considered in this study that the density of *P. pinaster* wood at the time of felling is equal to 1 g/cm^3, so the conversion of volume to biomass is direct and equal to 1, that is, 1 m^3 is equivalent to 1 ton, so the loss of volume (m^3) is equivalent to the loss of mass (t). This is accepted to be a simplification, but it does not alter the objectives pursued.

2.2 Definition of the premises for the study

It was assumed that the harvest will be done by the producer-owner of the forest and that the entire field operation will be its own.

Regarding the exploration model, scenarios of exploration of wood, resin and wood integrated with resin were considered. The scenario analysis period will be 10 years, considering year 1 (t1 = 35 years) and year 10 (t10 = 45 years). The initial age of 35 years was considered because at this age the simulated scenario indicated the predominance of trees with a diameter above 20 cm, a legal and favorable condition for resining.

The exploration model considers the premise that the forest did not undergo resining prior to the study period. In scenarios where there is logging, a thinning occurs every 5 years, which means that there are two thinnings during the simulation: at t5 (40 years) and at t10 (45 years). The trees to be removed in the thinning from below were those with the lower dimensions, both in height and diameter.

The characteristics of the target forest stand for the study are based on the average pattern of *P. pinaster* forests found in northern Portugal, where the relief is moderately sloping, with the presence of some rockiness. The stands, in general, have irregular spacing as they result from natural regeneration. It was assumed that spontaneous vegetation in the understory is low. The wood volumes are considered over bark.

The model of timber harvest adopted was that of services performed by the owner himself, since the removal of timber will be occasional. It was considered that the sale value of standing wood will support all forest maintenance costs, such as controlling spontaneous vegetation, maintenance of roads and firebreaks, inventory and combating pests and diseases, and will be deducted from its value by 30% considered as profit and taxes of the producer, with the remaining 70% added to the investment value for the production of wood delivered to the consumer.

In relation to exploration and transport operations, average utilization rates were always above 70%, high base productivities of machines and equipment when not impacted by the experience of the operator (which was adopted as 'experienced', which considers the potential to reach 100% of the expected productivity) and operating conditions, which was considered good for this study (low slope, few rocks, dry weather and non-aligned trees) because was considered that these conditions will not to influence the operating income. Income and operating cost were adjusted in accordance with the practice of a leading company in the Portuguese forestry market, through a personal report from its forestry engineer.

Regarding the costs related to the machines, the market acquisition values were considered for amortization in 60 months [25] at a residual value of 10%. Fuel consumption and other inputs, as well as maintenance and administrative costs, were obtained from personal information obtained from the field coordination of the company in question.

The description of the operating process for the production of resin, its costs and investments were collected through personal information provided by a technician specialized in the area.

Market values for standing wood delivered to the consumer and the market price of the resin and the rental price of the spouts were also obtained from the aforementioned company.

2.3 Methodology for defining biomass (t) and characteristics of available wood

To determine which interventions should occur in the forest stands and thus create the operational scenario for the harvest of the forest, the Modispinaster simulator [21, 26] available on the CAPSIS platform [27] was used. The simulator requires, as input data, information on basal area (G) of the stand, mean quadratic diameter (dg), dominant height (hdom), stand age and stand density (trees ha^{-1}). The simulator returns the output data (outputs) that refer to the forestry model that should be applied to the stand. The variable values needed to initializate the simulator were taken from Production Tables [20, 28]. A medium quality station (SI35 = 16 m) was assumed [29]. At the starting point, the pine stand was 15 years old and had a density of N = 2200 trees ha^{-1}, G = 12.8 m^2ha^{-1} and dg = 8.6 cm.

Projection of growth was simulated over a 50-year period, subject to thinning from below. The density regulation was based on specifying the proportion of trees removed periodically over time. The intervention regime adopted considers a total of six thinnings, one every 5 years, starting at age 20 and ending at 45 years of age. The proportion of trees removed varies from 20–30%, with the following sequence: 20% (t = 20 yr), 30% (t = 25 yr), 30% (t = 30 yr), 25% (t = 35 yr)), 25% (t = 40 yr) and 25% (t = 45 yr).

According to this model of density regulation, the stand reaches a value of 20 cm at 31 years old. Of the entire growth projection period, this case study focuses, as mentioned, on the range of 35–45 years. At 35 years of age, after performing a thinning, the trees are distributed in diameter classes (5 cm range) of 20 cm or greater.

2.4 Methodology for simulation of optimized wood production scenarios

The determination of the optimized scenario for the exploitation of wood by Modispinaster indicated the volume to be extracted per hectare so that production has is sustainable. Based on this volume value, operational scenarios for the harvest of wood were established. A programmed Excel sheet was used to generate monthly costs per ton in the exploration and transport of wood according to pre-established parameters. The sheet is not prepared to include the investment and cash flow values in the generation of costs, only for the calculation of operating costs. The payroll is programmed from seven cost sub-groups, namely:

- Equipment costs: this cost group includes the acquisition value of new equipment for use in forestry harvest, such as Harvester, Feller-Buncher, Skidder, Forwarder, Crane, Tractor-Grapple, Self-loading Tractor, Shutter, Chainsaw and Support vehicles (Pick up and light vehicles). In this same group, there is also a certain period of depreciation/amortization of the acquisition values (predefined for 60 months), which can be changed according to the intensity and mode of use of the equipment. It can also be defined a residual value for goods, which is set to zero, and insurance for machinery and vehicles.

- Maintenance costs: The costs of tools, collective protection equipment and maintenance equipment for the establishment of a workshop (fixed or field), costs with spare parts, wearing material and preventive/predictive maintenance are included in this field.

- Fuel and input costs: The updated prices of fuels (diesel and gasoline) and inputs (hydraulic oil) are allocated in this field. Low consumption inputs such as coolants or anti-freeze, chain oils and lubricating grease were not allocated in this field, as these costs are considered to be already present in the amount allocated to maintenance costs.

- Personnel costs: This group of costs includes the expected salary to be paid to employees (machine operators, mechanics, assistants, administrative assistants and managers). Also in this group are the values with food, lodging, labour exams, insurance, extra benefits, payments for production, uniforms and personal protection equipment.

- Costs with contributions and taxes on labour: This sheet includes amounts related to compulsory fees and contributions, provisions for extra wages, leaves and charges related to work. The allocated amounts are linked to the personnel cost group and automatically feed it.

- Administrative costs: The costs necessary for the management of the business are represented here, such as costs with fees and taxes related to the license and operation of the project, training and training, food, travel and accommodation for the management, extra vehicles, Information Technology and office material, costs of renting rooms and their expenses, technical consultancy.

- Project profit margin: It is previously established as 20% for outsourced projects, where the calculation of profit occurs on the provision of services and 0% for own projects where the calculation of profit occurs on the sale of the product (wood).

Assumptions and parameters were included, such as hypothetical project area (see justification below), volume to be harvested per hectare, slope, occurrence of rocky outcrops and stones, density of the under storey spontaneous vegetation, density of trees per hectare in the forest, average volume of trees, weather conditions, price of standing wood, distance and average transport speed. The entry of these data into the excel sheet enabled the next step, which was the entry of variable data that enabled the creation of the lowest operating cost scenario.

Variable data for creating of scenarios was: Exploration system and machines as well as their productivities, mechanical availability, operational efficiency, employees' salaries, costs of acquisition of vehicles and machines, work shift used (number of days in the month, hours a day and number of shifts per day), support and maintenance structure (personnel and equipment) and type of operation (own or outsourced). Each of these variables was adjusted in the model, until the lowest possible cost per cubic meter/ton of wood was found.

It should be noted that there were assumptions for the generation of the operational scenario, and any modification in one of these will alter the results presented. In brief, the operational scenario is based on the following characteristics:

a. Exploration area: the area chosen as the starting point is 100 ha, as this will gain some scale and remain coherent with the characteristic of northern Portugal where small properties predominate. The oscillation of this area will be addressed in the discussions that follow.

b. Terrain slope: Moderate or gentle undulating slope with a declivity of 3–8% was taken by default [30], since in the north of Portugal forests are always located in areas with some slope.

c. Spatial arrangement: A configuration with irregular tree spatial distribution was adopted as it is the most common situation and poses greater operational challenges.

d. Machine operator experience: It is assumed that the operators of the machines and equipment selected have experience and practice and have already gone through the entire learning curve in order to find themselves at the peak of productivity.

e. Weather: It is related to weather conditions; it is considered that the entire operation takes place in dry weather, without rain.

For the final analysis, considering the lower-cost scenario, it was assumed that the value of standing wood already includes all previous costs such as opening and maintaining roads, clearing vegetation of the land, planting, combating trees' pests and diseases, inventories and others. The sum of the cost of standing wood, plus the cost of exploration and transport, results in the final cost of the wood (delivered to the consumer).

By comparing the final cost of the wood with the value (price) paid by the market, the profit margin of the complete wood exploration process was obtained. From the scenario chosen for logging adjusted in the excel sheet, resin production revenues and expenses were generated for 0% (Scenario 1-W) and 100% of trees above 20 cm in diameter breast height (Scenario 2-MR) and after that, a third scenario was created to consider only resin production (Scenario 3-R). To define the amount of wood harvested, it was considered that there was a reduction in the volume of wood obtained in each scenario as a function of resin. Afterwards, this reduction was applied in the annual increment of the forest according to the percentage of tapped trees. It was considered that in the study area there is no history of resin tapping.

Table 2 specifies the verified exploration scenarios.

The creation of each exploration scenario allowed obtaining the unit cost for wood (€/t) and resin (€/kg), and by multiplying this cost by the quantity produced, the values of capital outflows from the cash flow were estimated. The multiplication of the quantities produced by the unit market value indicates capital inflows into the cash flow, essential elements for the financial feasibility analysis below.

Scenarios	Products	% of tapped trees (d > 20 cm)
Scenario 1 (W)	Wood	—
Scenario 2 (WR)	Wood + Resin	100%
Scenario 3 (R)	Resin	100%

Table 2.
Scenarios of wood and resin exploration in a Pinus pinaster forest.

2.5 Methodology for the analysis of financial feasibility

The financial feasibility analysis was developed based on four classic indicators for this type of analysis: The Internal Rate of Return (IRR), Net Present Value (NPV), Net Profitability Index or Return on Investment (NPI) and the period of recovery or time of return on investment (Payback) obtained through an Excel spreadsheet.

The selected indicators are the most frequently used for financial viability analyses. The internal rate of return (IRR) is the maximum rate that an investor can invest so that there is no capital loss. The net present value (NPV) is based on comparing the project's cash flows with the initial investment, and the project is said to be profitable when its value is positive, and the net profitability index (NPI) returns the effective return per unit of capital invested in the project while the recovery period or Payback indicates whether the invested capital is recovered in a shorter period than the projected time [31].

A viable project from a financial point of view is one that allows the return of the invested capital to the investor in a period of time shorter than the period of analysis (Payback), which is established in this study in 10 years, as it is compatible with long-term projects common in the forestry area. Profitability index above 1, which means that for each invested unit, one unit returns to the investor plus the percentage above 1 represented by the decimal places of the indicator.

The internal rate of return must be greater than the opportunity cost, which means that the project remunerates the invested capital above other possible investments considered, for this project the value of 5% per year was considered. The net present value must be positive, if we retroact the values considered in the cash flow (investment + inflows +outflows) to the present time, the value must be positive because values below zero indicate that there was a financial loss [31]. **Table 3** defines the parameters that will be considered for the final analysis.

To calculate the indicators, it was previously necessary to raise the investments and build the cash flow (CF).

For the investments, all costs of acquisition of vehicles, machinery and equipment provided for in the excel sheets were used for the preparation of the scenarios, plus the value of 3 months of personnel costs and consumable materials

Indicator	Reference Value	Interpretation
IRR	>5%	The bigger the better
NPV	>0	The bigger the better
NPI	>1	The bigger the better
Payback	<10 years	The smaller the better

Table 3.
Parameters for financial analysis.

Parameters	Description
Expected rate of return (K)	5%
Market price per ton of standing wood	€ 20.00
Cost per ton of standing wood (investment for production after thinning)	€14.00
Price per ton of pulping wood delivered to the final consumer (industry)	€ 40.00
Wood and resin exploration system	Own (acquisition of equipment and execution of services by the forest owner), free of taxes and profit.
Average distance of wood transport	10 km (forest/industry)
Price per kg of resin paid by the processing industry	€ 2.00
Resin production by spout	2 kg

Table 4.
Parameters adopted for analysis of financial feasibility.

as working capital. The definition of a working capital of 3 months considers that the project will have 1 month of preparation/mobilization, 1 month of production and 1 month of bureaucratic procedures for the first entry to occur capital (payment).

The cash flow is composed of capital inflows and outflows. The value of the entries was obtained by the annual quantity of wood for wood chips (the most representative product in the market) and/or resin produced, multiplied by the price paid by the market. Wood for veneer and sawmill may suffer loss of value due to stretch marks (wood damage) [32]; however, for this study, this loss was not considered because it was decided to simulate scenarios only of wood for cellulosic pulp and energy.

The output value was calculated by the amount of wood/resin produced multiplied by the cost (indicated by the companies) of production indicated by each scenario.

The assumptions used for the project feasibility analysis are shown in **Table 4**.

3. Results and discussion

3.1 Determination of wood productivity adjustment factor due to the impact of resin

The analysis of **Table 1** indicates potential diameter loss ranging between 8 and 33%, for heights losses between 6 and 12% and for volume losses between 0 and 20% for trees ranging in age from 23 to 140 years old. After excluding the values of the aforementioned articles incompatible with the study [16, 14], the stem volumes and the volume losses were estimated according to the methodology described in Section 2.1. The volume loss ranged between 0 and 20%, and the average value for the sequence of studies of 16% biomass reduction was adopted. The estimated rate of volume loss is in agreement with the value mentioned in literature for maritime pine in Portugal [15]. The differences found with other studies [16, 14] are expected because the losses of dimensions in the trees can be conditioned for genetics factors and environmental variables that change in different sites.

3.2 Definition of volume and characteristics of available wood

After processing the input data and simulating the growth of the stands and periodical silvicultural practices, the Modispinaster simulator returned the results that were taken as the initial parameters in establishing a model for the scenario feasibility analyses. **Figure 1a** and **b** represent the diameter distribution of the stand before and after thinning at 40 years of age. This moment represents the centre of the analyzed period (t5 = 40 years). At 40 years, the diameters at breast height are above 20 cm and the most represented class is that of 25 cm. The quadratic mean diameter changes from 25.9 cm before thinning to 27.0 cm after thinning with this variation being associated with the thinning from below.

In order to delimit the analysis scenario, it was decided to work with fixed values of the number of trees with breast height diameter above 20 cm before and after the intermediate thinning; therefore, no entry, neither loss of individuals was considered in the 5 years preceding and in the following thinning. For the pre-thinning scenario (t1–t5), the defined scenario indicates that the number of is 645 treesha^{-1}, and in the post-thinning scenario, it is 485 treesha^{-1}. These numbers will be used to define the mass of resin produced in each period.

Finally, the theoretical wood biomass to be extracted from the forest in the two-thinning scheduled to occur at 40 years (t = 5 years) and at the end of the analysis cycle, at 45 years (t = 10 years), are 37 tha^{-1} and 45 tha^{-1}, respectively.

3.3 Simulation of optimized wood and resin production scenarios

As already mentioned, there are three management scenarios to be analyzed: the exploration of wood (W), resin (R) and the integration of wood and resin (WR) exploration in the same area (**Table 2**). Initially, the wood and resin exploration scenarios will be analyzed separately, and later the integration of both products was carried out in order to consider the loss of 16% of wood due to resin coating.

3.3.1 Logging

The analysis system adopted initially indicated the lowest cost per ton of wood for a mechanized logging system (7.19€ha^{-1} of wood stacked on the road). However,

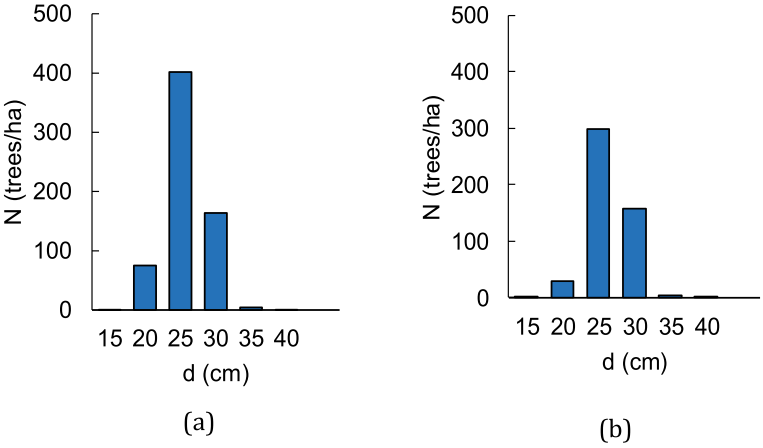

Figure 1.
Diameter distribution before (a) and after (b) thinning at 40 years old.

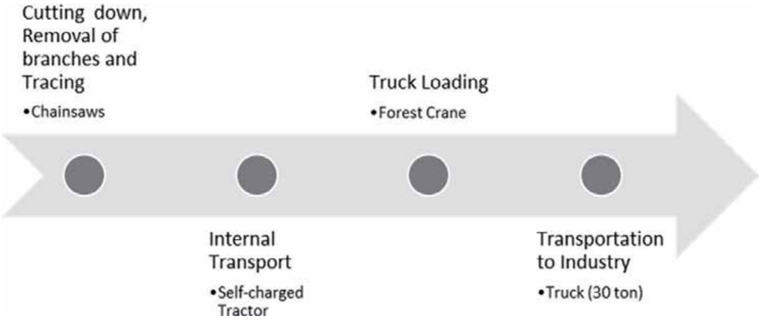

Figure 2.
Logging system (scenario W).

when analyzing the scenario from the perspective of the technical component, it should be considered that because it is a natural regenerated forest, the access to a trail by the equipment can be impossible due to the arrangement and density of trees on the ground. Therefore, the system was conservatively adjusted for the use of chainsaws, and the system was then designed as indicated in flowchart below (**Figure 2**)

The final cost for logging was 12.27 €ha^{-1} for harvesting and delivery operations and 5.64 €ha^{-1}of wood for transporting the forest to the consumer (industry). These costs are very different and sensibly smaller than the costs indicated for [7] that indicates generic values that were not adjusted for any particular case, therefore is important in deeper analysis to calculate the costs, using the [7] only for cases that do not require precision.

3.3.2 Resin exploration

The resin exploration system followed the traditional model adopted in Portugal, which occurs with essentially manual operation, where the worker in the field opens streaks that lead and converge the resin flow to a central point below the wounds, determining the so-called 'spout', where there is a container to collect the resin to be later transported to the storage area before going to the transformation industry.

The system was designed as shown in **Figure 3**.

In the resin production in Portugal, the producer finishes his actions when he delivers the resin in a stock location, and from there the buyer (industry) collects

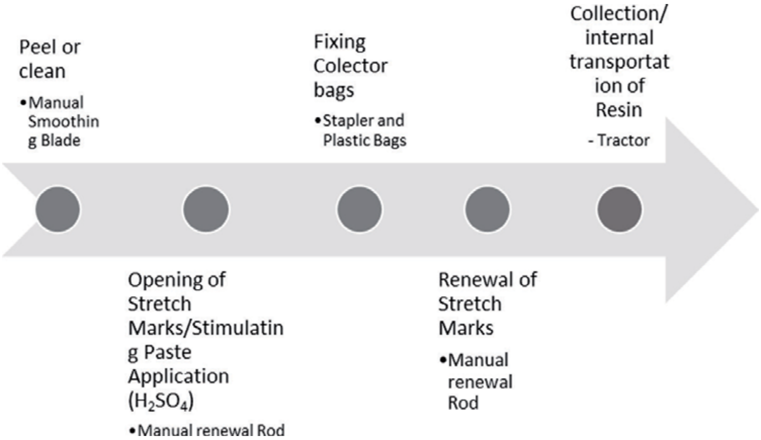

Figure 3.
Resin exploration system (R).

the resin and performs all the logistics to the plant, so actions such as stockpiling, truck loading, transportation and delivery are not indicated in the flow of the resin above.

In the model conceived in light of the characteristics of the forest and the operation in the field, the spreadsheet in Excel returned the cost of the resin at 1.06 €kg^{-1}, and this cost will be multiplied by the quantity produced for the composition of the outputs or expenses of this activity and, in turn, will participate in the formation of the cash flow.

3.3.3 Integrated wood and resin exploration

When resin production occurs in conjunction with resin production, that is, both products are explored in the same stand, it is expected that beneficial synergies to the process will occur, resulting in optimized operational and financial indicators. For example, a single manager can monitor both processes, and so there is a reduction in personnel involved and consequently their costs. Among the most likely synergies in this type of systems integration are the optimization of the operational workforce, the administrative system (management and control) and the use of machines and equipment. The mentioned synergy aforementioned was defined by [33], which explains when there is a production increase, using the same resources, occurs a dilution of fixed costs and the variable costs remain equal, so the final cost decreases.

For the subsequent analyses, in the WR scenario, such synergies or gains arising from the joint exploration of wood and resin were not considered, because, in the case of a simulation, the possible results of this exercise would be too broad and subjective. The number of scenarios that can be simulated is considerably large, and despite there were evidence that tapped trees can present anatomic differences from non-tapped trees [34], the WR scenario was designed in a simplified way by the sum of investments, costs and revenues of scenarios M and R separately. There are studies that confirm wood and resin exploration are viable, as [35] indicates this exploration as a way to recover the loss with thinning of small volume trees. The benefits from joined production of wood, resin and carbon may extend the optimal rotation age of Pine plantations [36].

3.4 Financial feasibility analysis

The values used to compose the cash flows resulting from the values simulated in each of the scenarios and that allowed the analysis of financial feasibility are presented in **Table 5** for 100 ha.

Table 6 shows the results obtained from the cash flow analysis for each indicator defined for the financial feasibility analysis.

The analysis of **Table 6** clearly indicates that the three scenarios, respecting the defined technical and operational conditions, are financially viable.

The Internal Rate of Return (IRR) is always greater than the predetermined rate of return of 5%. Scenario R presents the highest rate of return on invested capital (20.5%) followed by scenario W and WR, respectively, with similar rates of 12% and 11.9%. The excellent performance of the IRR indicator in the R scenario is due to the fact that in this situation, despite the absolute return values on the cash flow, the investment is very small when compared to scenario W and therefore to scenario WR. Scenario R has an excellent return; however, for larger gains in absolute values, a much larger scale of exploration (number of trees and area) is needed than the one fixed in the study. The advantage of scenario R was verified too in *Pinus elliotti* in Brasil, using a similar methodology, where the exploration of resin indicates a superior IRR than timber or timber and resin [37].

Cash flow	Stand Age (years)	W (€)	WR (€)	R (€)
Initial investment	35	−543,537.07	−566,604.22	−23,067.14
Cash Flow year 1	36	−20,000.00	−14,408.09	5591.91
Cash Flow year 2	37	−20,000.00	−14,408.09	5591.91
Cash Flow year 3	38	−20,000.00	−14,408.09	5591.91
Cash Flow year 4	39	−20,000.00	−14,408.09	5591.91
Cash Flow Year 5 (Roughing)	40	714,221.41	699,813.32	5591.91
Cash Flow year 6	41	−20,000.00	−16,877.40	5591.91
Cash Flow year 7	42	−20,000.00	−16,877.40	5591.91
Cash Flow year 8	43	−20,000.00	−16,877.40	5591.91
Cash Flow year 9	44	−20,000.00	−16,877.40	5591.91
Cash Flow Year 10 (Roughing)	45	723,556.63	746,679.23	5591.91

Table 5.
Cash flow in the three projects verified (values in euros, €/100 ha).

Indicator	W	WR	R
IRR	12.0%	11.9%	20.5%
NPV	317,894.46 €	325840.49 €	19,154.42 €
NPI	1585	1575	1830
DPB	FC 10	FC 10	FC5

Table 6.
Financial feasibility indicators for scenarios W, WR and R.

The Net Present Value (NPV) is positive for the three scenarios, and it is higher in the WR Scenario by an amount of 325840.49€, followed by the NPV of Scenario W of 317,894.46€ and finally a lower absolute value in Scenario R of 19,154.42€. The positive NPV is a good feasibility indicator only if we consider the isolated analysis of each scenario whether positive or negative. However, it should not be used as a comparative indicator, because it is a proportional number. That is, larger investments tend to generate higher NPV, as it is the case of scenarios W and WR which have investment values in the hundreds of thousands while scenario R has an investment in the tens of thousands of euros. The NPV was confirmed to be good indicator by [38], which indicates that exploration of timber and resin brings economics benefits to forest owners in United States. The same result was found for [36] in Java.

The Net Profitability Index (NPI) also indicates feasibility in the three scenarios and once again the NPI of Scenario R of 1.83 stands out, above the indicators of scenarios W, which presented an NPI of 1.585, slightly higher than the WR scenario, which presented an NPI of 1.575. Once again, in the same trend as the IRR, the low investment in Scenario R allows for a better performance of this indicator.

The Discounted Pay Back (DPB) or Recovery Period of invested capital is established at 5 years for the R scenario and 10 years for the W and WR scenarios. The best rates of return and net profitability index of scenario R, given the low investment, once again place this scenario with the best indicator.

4. Conclusions

Scenario R, where only resin is explored, has the best financial indicators and has less operational complexity; however, given the low absolute values of investment, the financial result also generates low absolute values, which means that for a businessman to bet a resin exploration project, it is necessary to gain scale (largest area and number of trees). Resin production can be scaled up by increasing the area, increasing productivity by spout or by increasing the number of trees harvested per hectare or by all both productions.

The integration between the wood and resin exploration that generates the WR scenario, which, despite being viable, generates the worst indicators of those presented, being very close, but still below the indicators of Scenario W. The reason for the worsening of the indicators is due to the fact that the 16% loss of wood volume/biomass that the resin imprints on the wood is not surpassed by the generation of resources from the resin.

The 10-year Payback of the scenarios where there is logging (W and WR) indicates that in the first thinning the producer does not recover his invested capital, which only happens in the second thinning at 10 years (corresponding to a stand age of 45 years, in the case study); however, with the gain of scale and use of the structure for projects that occur in parallel, this effect can be mitigated.

Increased productivity, increased sales price and gains in scale in the area significantly improve the scenarios presented. An increase of only 1 cent in the price per kg of resin already makes the WR scenario more attractive than the W scenario.

Finally, once this study was based on simulations, it is important that new studies may add precision in the results with a larger stand of data from field.

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Author details

André Sandim[1]*, Maria Emília Silva[2], Ana Cristina Gonçalves[3], Margarida Tomé[4] and Teresa Fidalgo Fonseca[1,4]

1 Department of Forestry Sciences and Landscape Architecture (CIFAP), Research Fellow RePlant Project, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

2 Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

3 Department of Rural Engineering, School of Sciences and Technology, MED - Mediterranean Institute for Agriculture, Environment and Development, Institute of Research and Advanced Education (IIFA), University of Évora, Évora, Portugal

4 Forest Research Centre (CEF), School of Agriculture University of Lisbon, Lisboa, Portugal

*Address all correspondence to: andre.sandim@utad.pt

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Chapter 8

Recent Megafires Provide a Tipping Point for Desertification of Conifer Ecosystems

Daniel G. Neary

Abstract

Recent megafires and gigafires are contributing to the desertification of conifer forest ecosystems due to their size and severity. Megafires have been increasing in their frequency in the past two decades of the 21st century. They are classed as such because of being 40,469 to 404,694 ha in size, having high complexity, resisting suppression, and producing desertification due to erosion and vegetation type conversion. Increasingly, gigafires (>404,694 ha) are impacting coniferous forest ecosystems. These were once thought of as only pre-20th century phenomena when fire suppression was in its infancy. Climate change is an insidious inciting factor in large wildfire occurrences. Fire seasons are longer, drier, hotter, and windier due to changes in basic meteorology. Conifer forests have accumulated high fuel loads in the 20th and 21st centuries. Ignition sources in conifer forests have increased as well due to human activities, economic development, and population demographics. Natural ignitions from lightning are increasing as a result of greater severe thunderstorm activity. Drought has predisposed these forests to easy fire ignition and spread. Wildfires are more likely to produce vegetation shifts from conifers to scrublands or grasslands, especially when wildfires occur with higher frequency and severity. Severe erosion after megafires has the collateral damage of reducing conifer resilience and sustainability.

Keywords: wildfire, megafires, gigafires, desertification, type conversion, drought

1. Introduction

Wildfires are now the most common disturbance in forest ecosystems other than tree harvesting [1]. Warmer, dryer, and windier weather conditions that are characterizing climate change-related drought in the western USA and elsewhere are driving wildfire occurrence and severity [2]. Future wildfire conditions are most likely to be aggravated in coniferous and boreal biomes, but grasslands are also at risk of serious disturbance [3]. Wildfire size and terrain features have also contributed to a destructive nexus of conditions that have resulted in unprecedented fire disturbances to wildland and urban landscapes. Forested catchments are particularly susceptible to this disturbance [4, 5].

Fire is not new to the planet. It has been a major disturbance force affecting terrestrial ecosystems since vegetation developed as an abundant fuel 450 million years during the Paleozoic Ordovician Period [6]. The sedimentary record indicates that wildfires have been occurring since the Paleozoic, but they increased substantially with the development of plant fuels in a lightning-filled atmosphere of the Carboniferous Period (307 to 359 million years before the present). Fire was one of the environmental and evolutionary pressures that created forest and grassland ecosystems. Humans then used fire as an ecological agent to further sculpt vegetation to suit their needs [6]. What was once a relatively stable and predictable tool for use in forest and grassland ecosystems, is now, under the pressure of changes in the climate and human activity, an unpredictable ecological stressor. Wildfires are now burning in meteorological environments that are hotter, windier, and drier than in previous decades [2]. The result has been on fires increasing numbers, size, severity, and complexity. Forest management has been forced to change to adapt to these conditions by placing more resources into fire suppression and management.

One example can be easily viewed in decadal areas burned by wildfire in the southwestern United States (**Figure 1**). Accurate wildfire records began tallying areas burned at the turn of the 20th century (1910). For the next eight decades, the cumulative area burned in each decade was steady with less than 20,235 ha (<50,000 ac) burned by wildfires that were small in areal extent. An ecological tipping point occurred in the 1990 to 1999 decade when the burned area doubled due to increasing numbers and size [7]. The next decade (2000 to 2009) saw a 69.3-fold increase. The following decade was characterized by an even larger 110.6-fold increase over the average of the 1910 to 1990 decades. In the first year of the 2020–2029 decade so far, wildfires burned over the record sizes of wildland landscapes.

A second example comes from Australia which suffered another devastating, record-smashing bushfire season in 2019–2021. Australia is no stranger to bushfires but climate change is wreaking havoc on the continent [8]. The 2019–2020 season proved to be unprecedented in many ways [9]. The first major bushfires began even before the official arrival of spring in June. Then, new out-of-control fires ignited at the beginning of Sept. 2019. This was followed by even worse fires at the beginning of November 2019 due to a lengthy drought and increasing temperatures. High temperatures, drought, and high winds in the late summer aggravated the bushfire escalated the crisis again over the first weekend in February. The fires in this outbreak were either extinguished or contained in early March after 9 months of

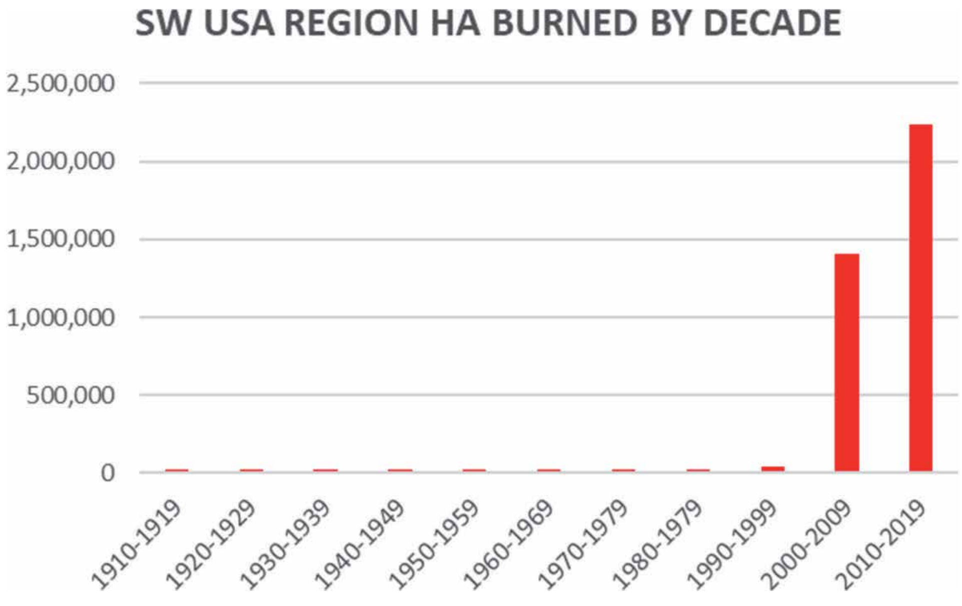

Figure 1.
Area burned in the southwest region by decade.

raging around the Australian continent. The infrastructure, ecosystem, and human impacts were staggering.

The bushfires burned more than 18.6 million ha, an area the same as the entire State of Washington in the USA, 70% of New Zealand, or 55% of Finland. At least 3500 houses and 5852 other buildings were burned to some degree. A total of 34 people died as a direct consequence of the bushfires between September 2019 and March 2020, but another 445 died due to other fire-related medical co-morbidities. This was much more than the 170 people estimate that died in the 2009 bushfires. Economic losses were initially estimated at the USA$1.3 billion in insured claims. But it may not be possible to completely determine the real economic loss from the bushfires because of: 1) the difficulties in evaluating intangible losses, 2) the confluence of the bushfires and COVID-19 impacts, 3) mortality of a billion native animals [10], and 4) the impacts on Australian fishing and tourism. The real economic effects probably surpass the infamous Black Saturday fires of 2009 that resulted in losses of the USA$2.9 billion to the Australian economy.

On the other side of the Pacific Ocean, the California wildfire season was record-setting with 9639 fires burning 1,779,730 ha [11]. Direct deaths were about the same as in Australia but there were ten times as many fatalities due to indirect air pollution impacts. The economic cost was much higher at over >$USA 12.1 billion. The number of buildings destroyed was 10,488 which contributed to the high cost. One gigafire, the August Complex, set the California record for size (417,907 ha) [7]. The 2021 fire season is underway and has the potential to eclipse 2020, which was a record year.

2. Wildfire, climate change, and drought

The trends in global wildfire potential under climate change was investigated by Liu et al. [12] using drought indices and general circulation models. It is shown that future wildfire potential increases significantly in the United States, South America, Central Asia, southern Europe, southern Africa, and Australia. Expected changes in drought and fire potential are expected to be the largest and smallest in southern Europe and Australia, respectively. The increased fire potential is mainly caused by warming in the U.S., South America, and Australia and by the combination of warming and drying in the other regions. The results of the Liu et al. suggest dramatic increases in wildfire potential that will require increased future investments in human resources, fire suppression infrastructure, and management activities to prevent fire disasters and recover from fire catastrophes. Stephens et al. [13] examined the role of drought-induced tree mortality in fueling wildfires. Their analysis points out that the scale of the western USA tree mortality creates a risk for even greater landscape-scale wildfires in the coming decades.

3. Wildfire characteristics

3.1 Ignition sources

The types of wildfire ignition are related to natural sources such as lightning, but more importantly human activities (e.g. agriculture, vehicle operations, and forestry activities), infrastructures (e.g. power lines, railways, etc.), or human behavior (e.g. recreation, delinquency, etc.). The main sources of human-caused ignitions vary by country but also at a regional scale [14, 15]. However, despite its importance in the improvement of fire prevention, knowledge of human-induced fire ignitions is still very limited in most parts of the world [16]. Ignitions by lightning are considerably enhanced by long-term drought plaguing forest regions of the world.

3.2 Frequency

Wildfire frequency is a key factor in describing a fire regime. It is a useful concept for comparing the relative role of fire between ecosystems and for describing the degree of departure from historical conditions [17, 18]. Brown [19] contains a discussion of the development of fire regime classifications based on fire characteristics and effects, combinations of factors including fire frequency, periodicity, intensity, size, pattern, season, and depth of burn, severity, and fire periodicity, season, and effects [20]. Several investigators have used modal severity and frequency to map fire regimes in the Western United States (**Table 1**) [21].

However, a number of the wildfire factors that affect this classification system have changed substantially in the past three decades. Wildfires are occurring over a longer period (season) and the fire climate is hotter, drier, and windier. This trend has been true for the past three decades and is accelerating. An example of the change in frequency and size of wildfires can be seen in the data from the southwestern USA.

Wildfire burned area tracking started in 1910. For the next eight decades, the total burned area remained under 20,000 ha **Figure 1**. Starting in 1990, fires began to occur at a higher frequency, size, and severity as the regional climate shifted into a mega-drought. Fires in 2020 and 2021, the current decade, are occurring at a record-setting pace.

3.3 Severity

3.3.1 Severity definition

At finer spatial and temporal scales the effects of a specific fire can be described at the stand and community level [2, 22]. The fire term is used to describe the ecological effects of fire severity. It describes both the degree of ecosystem disturbance and the amount of change in ecosystem components. Thus, severity integrates the damaging effect of both the heat pulse above ground and the energy transferred into the soil. In essence, it describes the amount of heat that is released by a fire that ultimately affects ecosystem functions. Fire severity is a good descriptive term that categorizes multiple ecosystem impacts [23]. The most important factors which determine the degree of fire severity are the fuel characteristics and the type of combustion. The amounts of flaming versus smoldering combustion that occur when wildland fuels are burned determine the degree of severity.

Wildfire literature is rife with confusion between the terms fire intensity and fire severity. A consistent distinction between the two terms has emerged in

Fire regime	Fire frequency (Years)	Fire type
I	0–35	Understory Fire
II	0–35	Stand Replacement
III	35–100	Mixed
IV	35 * 100	Stand Replacement
V	>200	Stand Replacement

Table 1.
Fire regime classifications according to Hardy [21].

the past three decades as fire science has improved and evolved. Fire managers trained in the science of fire behavior prediction systems now use the term fire intensity in a strict thermodynamic sense to describe the rate of energy released [24]. Fire intensity describes the rate of above-ground fuel consumption and, therefore the energy release rate [25]. It can be measured in thermodynamic terms of heat transfer per unit length of the fireline (kW m^{-1}) [2, 26]. The faster a mass of fuel combusts, the greater the fire intensity and the shorter the time that the soil is subjected to heat impact. Fast-moving wildfires typically do not produce complete litter combustion, whereas slower fires can completely combust the litter layer of soils. The rate at which energy can be transmitted through soils is restricted by the thermal properties of the mineral medium. As mentioned earlier, the duration of burning is critically important to the ultimate effect on soils [27].

Fire intensity is often related to the total amount of energy produced during the combustion process, but it is a measure of both small-scale prescribed fires as well as large-scale wildfires. Most energy released by the flaming combustion of above-ground fuels is transmitted upwards, not downward into the soil [28]. For example, Packham and Pompe [29] determined that only about 5 percent of the heat released by a surface fire occurs as heat pulses are transmitted into the ground. Therefore, fire intensity alone is not a good measure of the amount of fire-derived heat transmitted downward into the soil. Changes that occur in the physical, chemical, and biological properties of the soil are better indicators of heat transfer to the ground. For example, a high-intensity, and fast-moving crown fire will consume little of the surface litter because only a small amount of the heat energy released during the combustion of fuels is transferred downward to the litter surface [22]. In this case, the surface litter is identified as severe and presents as blackened, charred litter, but not completely consumed ash. Fire intensity can be quantitatively measured but fire severity can only be described (low, moderate, or high).

In wildfires in Alaska and North Carolina, fast-spreading crown fires were observed to completely consume the forest canopy but did not even scorch all of the surface fuels. However, if the fire also consumes substantial surface and ground fuels as a result of a longer residence time on a site, more energy is transmitted into the soil. Then, damage to the soil system is much greater. In such cases, a white or white-orange ash layer is often the only postfire material left on the soil surface [2, 30, 31].

Because the actual energy release of fire cannot be easily measured across a burned piece of land, the term fire intensity has limited practical application when evaluating ecosystem responses to fire. Increasingly, the term fire severity is used to indicate the ecosystem effects of fire on the landscape and its components [2, 31]. Fire severity was commonly used to describe the magnitude of negative fire impacts on natural ecosystems in the past. Wider usage of the term to include all fire effects is proposed. In this context, severity does not necessarily imply that there are negative consequences. Thus, a low severity fire may be discontinuous in nature, restoring and maintaining a variety of ecological attributes that are generally viewed as positive. For example, in fire-adapted longleaf pine (*Pinus palustris*) or ponderosa pine (*P. ponderosa*) ecosystems, fire is viewed as a necessary disturbance for maintaining the ecological characteristics of these forest types. In contrast, a high severity fire may be a dominant, albeit infrequent, disturbance in a non-fire adapted ecosystem. For example, in spruce (*Picea* spp.) forests fire is often a destructive disturbance. Frequent low severity fire is normal in a fire-adapted ecosystem. While all high severity fires may have some significant negative social and ecological impacts, only in the case of non-fire adapted ecosystems is the long-term functioning of the ecosystem significantly altered.

3.3.2 Fire severity classification

Judging fire severity solely on ground-based processes ignores the aboveground dimension of severity implied in the ecological definition of the severity of a disturbance. This is especially important because soil heating is commonly shallow even when surface fires are intense [22, 28]. Fire intensity classes were combined with the depth of burn (char) classes by Ryan and Noste [32] to develop a two-dimensional matrix approach to defining fire severity. Their system is based on two components:

1. An above-ground radiation and convection heat pulse associated with flaming combustion, and

2. A below-ground heat pulse due to conduction from smoldering combustion where duff is present, or radiation from flaming combustion where duff is absent on bare mineral soil.

Fire-intensity classes qualify the relative peak energy release rate (kW m^{-1}), whereas depth-of-burn classes qualify the relative duration of fuel combustion [2]. The concept of severity focuses on the ecological impacts of fire both above-ground and below-ground. Ryan [22] revised the Ryan and Noste [32] surface fire characteristic classes and depth of burn classes. By this nomenclature change, two burned areas would be contrasted as having had, for example, an active spreading-light depth of burn fire versus an intense-moderate depth of burn fire, common in high severity wildfires (**Figure 2**).

3.4 Wildfire size: Megafires and Gigafires

Wildfires burn on a number of scales between and within wildfires. Most do not go beyond the Zone of Prescribed Fire (4 to 400 ha) or the low end of Small Wildfires (400 to 4040 ha) (**Table 2**). All large fires will have components of smaller-scale fires embedded within them. A change that has occurred in the past

Figure 2.
High severity wildfire impacts on a young Pinus ponderosa stand, after the 2000 rodeo-Chediski fire, apache-Sitgreaves National Forest, Arizona.

Fire size class	**Fire burned area range**	**Fire name**	**State prov.**	**Actual fire size**
	ha			**ha**
Micro	10^{-4}	"A Burning Stump"		
Zone of Prescribed Fires				
A	<0.1			
B, C, D, E	121 to 404			
Zone of Small Wildfires				
F G	404 to 2023 2023 to 4049			
H	4049 to 20,234	Schultz Fire 2010 Cerro Grande Fire 2000	AZ NM	6100 19,425
I	20,234 to 40,469	Okanagon Park 2003	BC	25,600
Zone of Megafires				
J	40,469 to 202,347	Rim Fire 2013 Chelaslie River 2014 Rodeo-Chediski 2002	CA BC AZ	104,135 133,098 189,655
K	202,347 to 404,694	Wallow Fire 2012 Biscuit Fire 2002 Dixie Fire 2021	AZ CA CA	217,741 229,057 384,150
Zone of Gigafires				
L	>404,694	August Complex 2020	CA	417,907
		Taylor Complex 2004	AK	428,500
		Yellowstone Fire 1988	MT/ID	607,042
		Peshtigo Fire 1871	WI/MI	1,214,083
		Great Fire 1910	ID/MT	1,600,000

Fire size class	Fire burned area range	Fire name	State prov.	Actual fire size
	ha			ha
		Miramichi Fire 1825	NB	1,700,000
		Chinchaga Fire 1950	BC/AL	2,000,000
		Victoria Black Fri. 1939	AUST	3,000,000

Table 2.
Modified wildfire size classes and individual fire examples (from [33, 34]).

three decades is the increasing number of wildfires and the scale of those fires. Mega Fires (4060 to 40,469 ha) are now more common and there is a resurgence of Giga Fires (>404,604 ha) [33, 34].

The largest Giga Fires known in the historical record are from the 19th and 20th Centuries when fire suppression knowledge, technology, and resources were limited or non-existent. Land managers and owners relied on weather changes to dampen fire activity. Both Giga and Megafires (classes J, K, and L) are more prevalent in the first two decades of the 21st Century due to fuel loadings and climate change. Wildfires are burning in hotter, drier, and windier weather conditions than was experienced in much of the 20th century. The sizes and severities of current wildfires are proving to be much more resistant to suppression activities. Consequently, the infrastructure, ecological, and economic costs continue to escalate.

4. Erosion

4.1 Types of fire induced erosion

Erosion involves three separate processes that are a function of sediment size, transport medium (water, wind, or air), and velocity. These are (1) detachment, (2) transport, and (3) deposition. Erosion occurs when sediments are affected by water, wind, or air and velocities that are sufficient to detach and transport sediments. Erosion is a natural process occurring on landscapes at different rates and scales depending on geology, topography, vegetation, and climate. Natural rates of erosion vary from <0.01 to 15.00 Mg ha^{-1} [2, 31]. These rates increase as annual precipitation increases, peaking in semiarid ecoregions on the transition desert to wet forest [35]. This occurs because there is sufficient rainfall to produce erosion from the sparser desert and semiarid grassland covers. As precipitation increases, the landscapes start supporting dry and eventually wet forests, which produce increasingly dense plant and litter covers that decrease natural erosion. However, if landscapes are denuded by disturbances (e.g. fire, grazing, timber harvesting, mining, and so forth), then erosion continues to increase with greater precipitation. Surface physical conditions, topography, and soil hydrological status after wildfires and prescribed fires are important for determining post-fire water flows and the magnitude of erosion (**Table 3**).

Apart from the consumption of vegetation, erosion is certainly the most visible and dramatic impact of fire. Wildfire suppression, prescribed fire, and post-fire watershed rehabilitation also affect erosion processes in wildland ecosystems. Fire management activities such as fireline construction, temporary roads, and new and unpaved roads receiving heavy vehicle traffic will increase erosion. Stormflows after wildfires will also accelerate erosion rates. Burned Area Emergency Response (BAER) activities on watersheds have the potential to decrease some post-fire erosion

Soil surface condition	Infiltration	Runoff	Erosion
Litter Charred	High	Low	Low
Littter Consumed	Medium	Medium	Medium
Bare Soil	Low	High	High
Water Repellent	Very Low	Very High	Severe

Table 3.
Soil surface conditions that affect infiltration, runoff, and erosion after wildfires and prescribed fires (from [31]).

to varying degrees depending on the timing, amount, and intensity of rainfall, slope, degree of litter combustion, and the presence of water repellent soils [36].

4.2 Sheet, rill, and gully erosion

In sheet erosion, slope surfaces erode somewhat uniformly. This type proceeds to rill erosion in which small, linear, rectangular channels cut into the surface of a slope. Further redevelopment of rills leads to the formation of deep, large, rectangular to v-shaped gully [35]. Another type of slope erosion called dry ravel is initiated by a variety of disturbances, including fire. Dry ravel may best be described as a type of dry grain flow. Fires greatly alter the physical characteristics of hillslopes, stripping them of their protective cover of vegetation and organic litter, and removing log barriers that were naturally trapping sediment. Consequently, during and immediately following fires, large quantities of surface material are released and transit downslope as dry ravel even before rainfall events occur [37]. Dry ravel can equal or exceed rainfall-induced hillslope erosion after a fire in semi-arid ecosystems [38]. In the Oregon Coast Range of the United States, prescribed fires in heavy slash after clearcutting produced non-cohesive soils that were less resistant to the force of gravity [39]. Sixty-four percent of post-fire erosion occurred as dry ravel, not water erosion, happening within the first 24 hours after the end of active fire behavior.

4.3 Mass failure erosion

Mass failure erosion includes slope creep, falls, topples, rotational and translational slides, lateral spreads, debris flows, and complex movements. The largest, most dramatic, and main form of mass wasting that delivers sediment to streams are debris flows [40]. Most fire-associated debris flows are associated with the development of water repellent conditions in soils [2]. These mass failures are a large source of localized sediment delivered to stream channels. They can account for 50% of the total post-fire sediment yield in some ecoregions). Wells [41] reported that wildfire in chaparral vegetation in coastal southern California can increase average sediment delivery in large watersheds from 7 to 1910 $m^3\ km^{-2}\ yr^{-1}$. However, individual storm events in smaller basins can produce much greater sediment yields. Single storms have delivered sediment yields as high as 65,238 $m^3\ km^2$ in unstable terrain.

4.4 Channel destabilization erosion

Fire-related sediment yields depend on fire frequency, climate, vegetation, and geomorphic factors such as topography, geology, and soils [41, 42]. In some regions, more than 60% of the total landscape sediment production over the long term is fire-related. Much of the sediment production can occur the first year after a wildfire [2, 43]. However, a risk of increased sediment in streamflow can persist for 10 or more years after a wildfire. Sediment transported from wildfire scars as a result of increased stream peak flows can adversely affect aquatic habitat, recreation areas, roads, buildings, bridges, and culverts. Management of newly deposited sediments is a problem in both the terrestrial and aquatic environment since fire-derived material can block roads, block culverts, alter drainage patterns, and fill in channels, lakes, and reservoirs [44, 45] (Reid 1993, Rinne 1996).

4.5 Effect of water repellent soils on post-fire erosion

Fire affects rainwater infiltration in two ways. First, the combustion of soil organic horizons leaves mineral soil unprotected from raindrop impact. The force of rainfall loosens and disperses fine soil and ash particles, causing the soil surface to seal [46]. Second, soil heating during a fire frequently produces a water-repellent layer at or near the soil surface. This process further impedes water infiltration into the soil. The severity of this water repellency in the surface mineral soil layer, however, decreases over time as it is exposed to moisture, freeze–thaw cycles, and animal and insect burrowing. In many cases, water repellency does not substantially affect infiltration beyond the first year. However, fire-induced repellency can persist for several years. Water repellency has a particularly important effect on two post-fire erosion processes, raindrop splash, and rill formation.

The sequence of rill formation as a result of fire-induced water repellency has been documented to follow several well-defined stages [2]. First, the wettable soil surface layer, if present, is saturated during initial infiltration. Water moves rapidly into the wettable surface ash layer until it encounters a water-repellent layer. This process occurs uniformly or discontinuously over the burned landscape so that when the wetting front reaches the water-repellent layer, it can neither drain downward nor laterally. If the water repellent soil layer is right at the soil surface, runoff starts immediately after rain droplets reach the soil surface. As rainfall continues, water fills all available pores until the wettable soil layer becomes saturated. Because of the underlying water-repellent layer, the saturated pores cannot drain, which creates a positive pore pressure above the water-repellent layer. The shear strength of the soil mass declines and it results in a failure zone located where pore pressures are greatest, at the boundary between the wettable and water-repellent layers. As the water flows down this initial failure zone, turbulent flow develops, which accelerates erosion and entrains particles from both the wettable ash layer and the water-repellent layer. The downward erosion of the water-repellent rill continues until the water-repellent layer is eroded away and water begins infiltrating into the underlying wettable soil. Flow then diminishes, turbulence is reduced, and down-cutting temporarily ceases. The result is a rill that has stabilized immediately below the water-repellent layer. Additional rainfall over time will cause these rills to deepen and widen into a gully network. On a watershed basis, these individual rills and gullies develop into a well-defined drainage network that can extend throughout portions of small and large watersheds. The net result is a dramatic increase in the volume of hydrologic response and a decrease in the timing of runoff from the catchment area.

4.6 Post-fire sediment yields

Natural erosion rates for undisturbed forests range from <0.01 to 7 Mg ha^{-1} yr^{-1} [2, 47], but do not approach the average upper limit of geologic erosion in highly erodible or mismanaged soils (560 Mg ha^{-1} yr^{-1} [48]. These differences are due to natural site factors such as soil and geologic erosivity, rates of geologic uplift, tectonic activity, slope, rainfall amount and intensity, vegetation density, and percent cover. Normal landscape-disturbing activities such as agriculture (560 Mg ha^{-1} yr^{-1} [49], mechanical site preparation (15 Mg ha^{-1} yr^{-1} [50], and road construction (140 Mg ha^{-1} yr^{-1}) produce a range of sediment losses.

Sediment yields from fires vary considerably, depending on fire frequency, climate, vegetation, and geomorphic factors such as topography, geology, and soils [2, 51]. In some regions, over 60% of the total landscape sediment production over the long term is fire-related. Much of that sediment loss can occur the first year after a wildfire but may extend to 10 years or more [2, 38, 43]. Sediment yields 1 year after prescribed burns and wildfires range from very low, in flat terrain and in the absence of major rainfall events, to extreme, in steep terrain affected by high-intensity thunderstorms. Erosion on burned areas typically declines in subsequent years as the site stabilizes, but the rate of recovery varies depending on fire severity, vegetation recovery, climate, and depth of soil loss.

Soil erosion following fires has been measured to range from under 0.1 Mg ha^{-1} yr^{-1} to 15 Mg ha^{-1} yr^{-1} in prescribed burns, and from <0.1 Mg ha^{-1} yr^{-1} in low severity wildfire, to more than 369 Mg ha^{-1} yr^{-1} in high-severity wildfires on steep slopes [2, 43, 50]. More recent analyses have estimated sediment losses after wildfires in steep terrain of upwards of 1500 Mg ha^{-1} yr^{-1} from a combination of steep slopes and high-intensity rainfall. Nearly all fires increase sediment yield, but wildfires in steep terrain produce the greatest amounts, >1500 Mg ha^{-1} yr^{-1} [52]. Sediment yields usually are usually the highest during the first year after a fire and then decline in subsequent years. However, if precipitation is below normal, the peak sediment delivery year might be delayed. In semiarid areas, postfire sediment transport is episodic in nature, and the delay may be longer. All fires increase sediment yield, but it is the combination of steep slopes, high severity fire, and intense rainfall that is the most problematic.

There is increasing evidence that short-duration, high-intensity rainfall (>50 mm h^{-1} in 10–15 minute bursts) over areas of about 1 km^2 often produces flood flows that result in large amounts of sediment transport [31]. *A* thunderstorm after the 2010 Schultz Fire in Arizona had a peak rainfall of 24 mm in 10 minutes and resulted in debris flows and floods that had a return period of >1000 years [52]. High severity fire (>70% coverage), steep slopes (>100%), and intense rainfall contributed to the unusual erosion. Best Management Practices certainly have value in reducing sediment losses from prescribed fires. However, mitigative techniques for reducing sediment losses after wildfires often that are often used as part of burned area emergency watershed response (BAER), have their limitations and cannot really cope with large erosion events.

After wildfires, streamflow turbidity usually increases due to the suspension of ash and silt-to-clay-sized soil particles [53]. Turbidity is an important water quality parameter because high turbidity reduces municipal water quality and can adversely affect fish and other aquatic organisms. It is often the most easily visible water quality effect of fires [2]. Less is known about turbidity than sedimentation in general because it is difficult to measure, highly transient, and extremely variable. Extra coarse sediments (sand, gravel, boulders) transported off of burned areas as a consequence of increased storm peak flows can adversely affect aquatic habitat, recreation areas, and reservoirs. Deposition of fine sediments as well as the previously mentioned coarse sediments destroys aquatic and riparian habitat, reduces the storage capacity of lakes and reservoirs, negatively affects stream and lake biota, degrades water quality, and imperils infrastructure [2, 45].

5. Desertification

Desertification was introduced into the fire-related lexicon in the 1940s by [54] before the modern outbreak of large fires. Although there is no general agreement on the definition of the term it is not necessarily associated with a classical desert. It is a landscape deterioration process that involves reductions of plant and soil ecosystem services. Desertification occurs on a continuum and is usually associated with human activities, especially erosion. The loss of key plant species and diversity, and erosion perturbation of soil physical properties and functions are key factors in the progression of desertification. The environmental hazards that result are most notably losses of soil fiber and food production capability, declines in water supply capability of watersheds [55], accelerated erosion of key soil horizons, and vegetation type conversions.

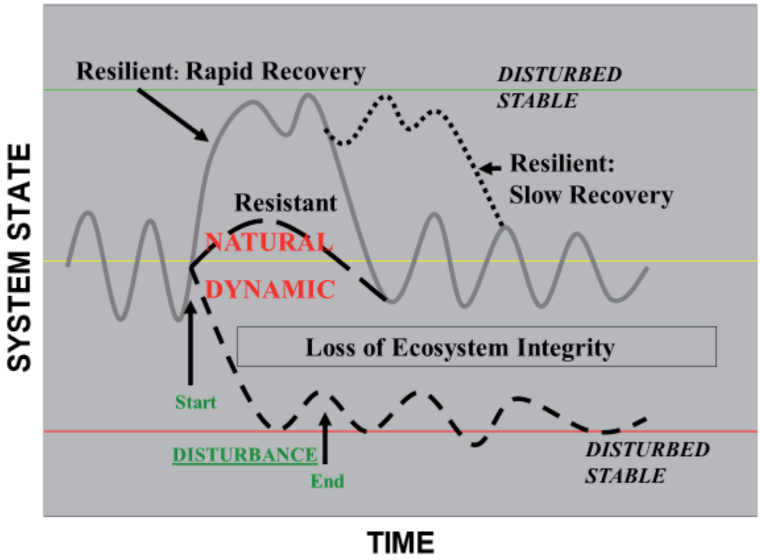

Figure 3.
Ecosystem responses to disturbance: Resistant, resilient, loss of ecosystem integrity.

The desertification process involves a shift in the normal ecosystem dynamic to a lower disturbed, but stable state (**Figure 3**). Fire-resistant forest ecosystems are characterized by a natural variability that stays within a normal range of disturbance and recovery. Fire resilient forests are disturbed from their normal range of variability but they recover rapidly or slowly. Excessive wildfire disturbance that results in the loss of ecosystem integrity pushes a forest to a lower system state that may never recover or take excessively long periods of time to do so [56].

6. Type conversion

Type conversions of ponderosa pine to chaparral scrublands is an example of loss of ecosystem integrity. Vegetation conversion from stable conifer forests to fire-prone scrublands usually produces an increase in fire frequency and severity which prolongs ecosystem persistence at a lower, desertified system state (**Figure 3**). Under these conditions, desertification magnifies the impact of the fire scale and the persistence of disturbance plant species [57, 58]. These investigators clearly point out the role of fire severity in driving plant community-type conversions. Keeley [58]. The greatest threat to the persistence of native California vegetation types is type conversion to herbaceous species more resilient to and more conducive to frequent fires. These fires are more likely to impact conifer species and prevent the re-colonization of severely burned sites [59]. Since 1996, high-severity crown fires in Southwestern ponderosa pine forests have produced large treeless areas, which are unprecedented in the regional historic record [60]. Other dry conifer forests, similar to ponderosa pine, are also experiencing extensive levels of high severity fire and type conversions to grasses and fire-prone scrub species.

7. Conclusion

It is clear now at the beginning of the 21st Century that changes in the climate have accentuated fire weather. Fires are now burning in hotter, drier, and windier conditions than they were 30 years ago. Wildfires are also burning into higher elevations, due to a warming climate. This climate condition has led to larger and higher severity wildfires since fires are more difficult to suppress and contain safely in steep terrain. In addition, fire seasons are now 4 months longer. In some areas, such as California, the fires season is 12 months long. This fire situation has provided an ecological tipping point leading to accelerated desertification of conifer ecosystems. This condition limits the success of management interventions to reverse desertification.

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Conflict of interest

The authors declare that there are no conflicts of interest related to the subject of this paper.

Author details

Daniel G. Neary
USDA Forest Service, Rocky Mountain Research Station, Flagstaff, Arizona, USA

*Address all correspondence to: danneary46@yahoo.com

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Chapter 9

Respiratory Allergy to Conifers

Denis Charpin, Hélène Sénéchal and Pascal Poncet

Abstract

The conifers, from the latin meaning “cone carrier,” include about 650 species distributed in seven families. They are found all over the world, and the most known conifers are the cypresses, the junipers, the yews, the larches, the firs, or the pines. The most allergenic pollen is emitted by the Cupressaceae/Taxaceae family with mainly five different genera: *Cupressus, Hesperocyparis, Juniperus, Cryptomeria*, and *Chamaecyparis*. The symptomatic period starts in November and ends in April. In Mediterranean areas, *Cupressus sempervirens* is the most common pollinating species. Five main cypress allergens have been thoroughly described. Depending on the geographic area and the studied population, the prevalence of cypress allergy in the general population ranges from 0.6% to 3%, and 9–65% of outpatients consulting an allergist are sensitized to cypress pollen. This prevalence is increasing likely to be due to the modifications of the environment. Rhinitis is the most prevalent clinical symptom, while conjunctivitis is the most disabling. Clear-cut improvements of the quality of life are observed upon an effective and safe specific immunotherapy. Associations with food allergy based on molecular allergen cross-reactivities were described resulting in sometimes severe symptoms. Pollens from Pinaceae family, especially pines or firs, although abundant, do not demonstrate a significant clinical impact.

Keywords: cypress pollen, pine pollen, allergens, aerobiology, epidemiology, botanic, clinic

1. Introduction

Respiratory allergic diseases are among the most prevalent chronic disease, affecting 20–25% of the general population. Allergy reactions at large encompass several mechanisms, but allergy reactions to pollen are considered as a “ type-1 ” or “ immediate-type ” or “ IgE-dependent ” hypersensitivity reaction involving mast cells and basophiles as effectors cells. Those cells are responsible for releasing inflammatory and immune mediators leading to ocular, nasal, and bronchial symptoms. Pathophysiology of these reactions allows the use of skin tests and/or measurement of serum specific IgE as powerful diagnostic tools.

The prevalence of allergy is increasing whatever is the allergenic source, pollen, food, animals. Pollen grains are the main inducers of respiratory allergies, and conifers play a major role around the Mediterranean basin, in North America, or in Japan. According to a phylogenetical classification, the conifers consist of one class, Pinopsida, and seven families have been described: Araucariaceae, Podocarpaceae, Sciadopityaceae, Cupressaceae/Cephalotaxaceae/Taxaceae, and Pinaceae. No extensive studies were reported on the allergenicity of the pollen grains from Araucariaceae and Sciadopityaceae, but a huge amount of data are published for

Cupressaceae/Taxaceae (also reviewed in [1]) and Pinaceae pollen (see below). Cephalotaxaceae are sometimes included in Taxaceae and Araucariaceae and might be assimilated to pine because of the Wollemi pine discovered in Australia. A few data are available on the allergenicity of Podocarpaceae pollen [2, 3].

This review provides an update on various aspects of the highly allergenic family of conifer, i.e., Cupressaceae (Chapter 2) with, first, a botanical and palynological presentation of cypress followed by the various cypress pollen allergens involved; second, data on epidemiology; and third, the clinical aspects together with the management of cypress pollen allergy. Chapter 3 is devoted to the poorly allergenic conifer family, Pinaceae.

2. Cupressaceae

2.1 Trees, pollen, and allergens

2.1.1 Trees

Cupressaceae corresponds to a family of the order Pinales. According to a phylogenetical classification, the family includes about 140–160 species with 27–30 genera. Cupressaceae is the most widely distributed conifer worldwide, except Antarctica devoid of any trees (**Figure 1**). Cupressaceae, commonly named cypress, is the most well-known gymnosperm family that produces allergenic pollen. Two main contributors to cypress pollen allergies belong to Cupressoideae by species from the *Cupressus*, *Juniperus,* and *Thuja* genera and to Taxodioideae by species from *Cryptomeria* and *Taxodium* genera [4] (see below the description of the respective allergens in the section "Allergens").

Besides botanical and phylogenetical classification, a classification was proposed based on the functional and structural aspects of allergens (**Table 1**) [4, 5]. These allergens in different species exhibit a high degree of homology, up to 97% between *Hesperocyparis arizonica* (Cup a 1) and *Cupressus sempervirens* (Cup s 1), although molecular studies led to a split of the two species into two different genera, *C. arizonica* being assigned to the newly created *Hesperocyparis* genus [6]. Botanical proximity is responsible for cross-reactivities. The same molecular-type allergen produced by botanically distant plants appears very limited [7, 8].

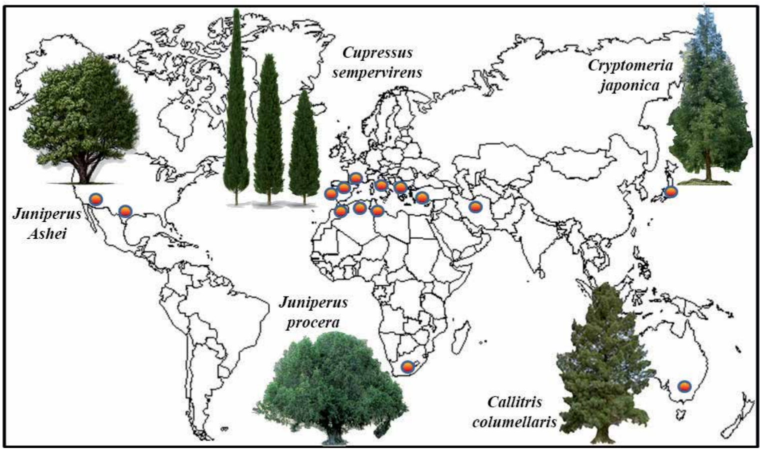

Figure 1.
Worldwide distribution of reported Cupressaceae pollen allergy (orange dots).

Groups	**Proteins**	**Cupressus sempervirens**	**Hesperocyparis arizonica**	**Cryptomeria japonica**	**Juniperus**		**Chamaecyparis obtusa**	**Taxodium disticum**	**Thuja**
					ashei	*other*			
		Italian cypress	Arizona cypress	Japanese cedar	Mountain cedar		Japanese cypress	Bald cypress	cedar
Group 1	Pectate lyase (40-45 kDa)	Cup s 1*	Cup a 1*	Cry j 1*	Jun a 1*	Jun c 1, o 1, v 1*	Cha o 1*		Thu p 1
Group 2	Polygalacturonase (43-60 kDa)	Cup s 2*	Cup a 2	Cry j 2*	Jun a 2*		Cha o 2*	Tax d 2	
Group 3	Thaumatin-like protein PR-5 (24-34 kDa)	Cup s 3*	Cup a 3	Cry j 3	Jun a 3*	Jun r 3, v 3*			Thu oc 3
Group 4	Ca-Binding protein (17-29 kDa)		Cup a 4	Cry j 4		Jun o 4*, v 4			
Group 5	Gibberellin-regulated protein (8kDa)	Cup s 7*	Cup a 7	Cry j 7*	Jun a 7*				
OTHER		ß-galactosidase 46-50 kDa	ß-galactosidase 46-50 kDa	Chitinase 27 kDa			Cha o 3* 63 kDa		
		Profilin (Cup s 8) 14 kDa	LTP 14 kDa	CJP8 (LTP) 17 kDa					
		Phenylcoumaran reductase 33 kDa		Isoflavone reductase 35 kDa					
		Rab-like protein 18 kDa		Aspartic protease 42 kDa					
		Sigma factor regulation protein 29 kDa		Serine protease subtilisin-like 79 kDa					

Groups	Proteins	Cupressus sempervirens	Hesperocyparis arizonica	Cryptomeria japonica	Juniperus		Chamaecyparis obtusa	Taxodium disticum	Thuja
					ashei	*other*			
		Italian cypress	Arizona cypress	Japanese cedar	Mountain cedar		Japanese cypress	Bald cypress	cedar
		Cytochrome c 12 kDa							
		SOD 15 kDa							
		Lactoyl glutathione lyase 32 kDa							
		Malate dehydrogenase 31 kDa							
		Triosephosphate isomerase 33 kDa							
		Glucanase 37 kDa							
		HSP104 104 kDa							

**referenced in IUIS/WHO database; Jun c:* Juniperus communis *(Common juniper) ; Jun o:* Juniperus oxycedrus *(Prickly juniper); Jun r:* Juniperus rigida *(Temple juniper); Jun v:* Juniperus virginiana *(Eastern red cedar); Thu p:* Thuja plicata *(Western red cedar); Thu oc:* Thuja occidentalis *(Eastern white cedar); SOD: Superoxide dismutase; LTP: Lipid transfer protein; HSP: Heat shock protein.*

Table 1.
Cupressaceae allergens. Name, protein function, and molecular masses (kDa) are indicated.

2.1.2 Pollen

2.1.2.1 Pollination: phenology and pollen features

Within a species, the pollination period is usually short. However, because of the different species in Cupressaceae, the pollination periods do not overlap, and this contributes to extend the exposition duration to Cupressaceae pollen from autumn to spring. In Mediterranean regions, pollen from early pollinating species (e.g., *Juniperus oxycedrus*) is produced in October or November [9–11], while pollination by late pollinating species (e.g., *C. sempervirens*) can occur up to late April or May [12]. Belmonte *et al* reported the diversity, floral phenology, and distribution of the Cupressaceae species in the Iberian Peninsula in 1999 [13].

All Cupressaceae species produce spherical pollen grains very similar in appearance (**Figure 2A**) [14]. In the *Cupressus* genus, the mean size of hydrated pollen grains varies from 25 to 40 micrometers (**Figure 2B**). However some variations can occur. Pollen grains are small for Mediterranean species (except for *C. dupreziana*, which produces diploid pollen [16], intermediate for New World species, and larger for Asian species [17]. The Cupressaceae pollen is inaperturate, although a faint circular pore blocked with a bulge can be seen in fresh material. The exine (outer membrane) is very thin and covered with scattered granules or orbicules (Ubish bodies, 300–600 nm) (**Figure 2A**). The intine (inner membrane) is very thick, and hydration unblocks the bulge leading to the swelling of the intine until the exine cracks (**Figure 2B**).

Cupressaceae trees are anemophilous, and pollen grains can be wind-transported over long distances because of their small size. Cupressaceae species generally produce huge quantities of pollen. The number of pollen grains per male inflorescence average 400,000, and production by individual trees has been estimated to be 276,000 million [18, 19]. Cupressaceae pollen predominates in the winter period, but can also be present all year long (**Figure 3**). In Mediterranean regions, *Cupressus,* together with *Olea,* produces the largest amount of allergenic tree pollen [20]. Cupressaceae/Taxaceae pollen is one of the 12 most abundant aero-allergenic pollens in Europe [21]. *Cupressus* pollen can account as much as 40% of the total annual pollen counts in Marseille, in Southern France [22], 38% in Antalya [23], and 35% in Istanbul, Turkey [24], 25% in Thessaloniki, Greece [25], 23% and 24% in Toledo and Cuenca, Spain [9, 26], 18% in Nicosia, Cyprus [27], 17% in

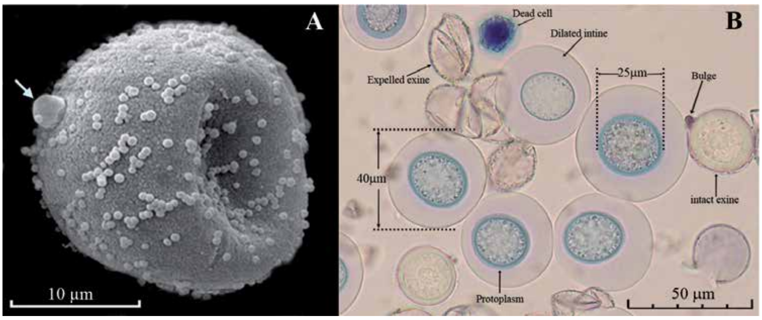

Figure 2.
A: Hesperocyparis arizonica *pollen grains (scanning electron microscopy from Shahali et al. [14]) collected in Tehran, Iran. (2225x magnification). The arrow shows the presence of a bulge on the external surface of the exine serving as a valve for regulating the entrance of fluids at the beginning of pollen hydration [15]. Numerous submicronic orbicules (300–600 nm) are visible on the pollen surface. B:* Hesperocyparis arizonica *pollen hydrated for 5 min in phosphate buffer saline. Optical light microscopy observation after viable trypan blue staining (100x magnification). The various elements are indicated.*

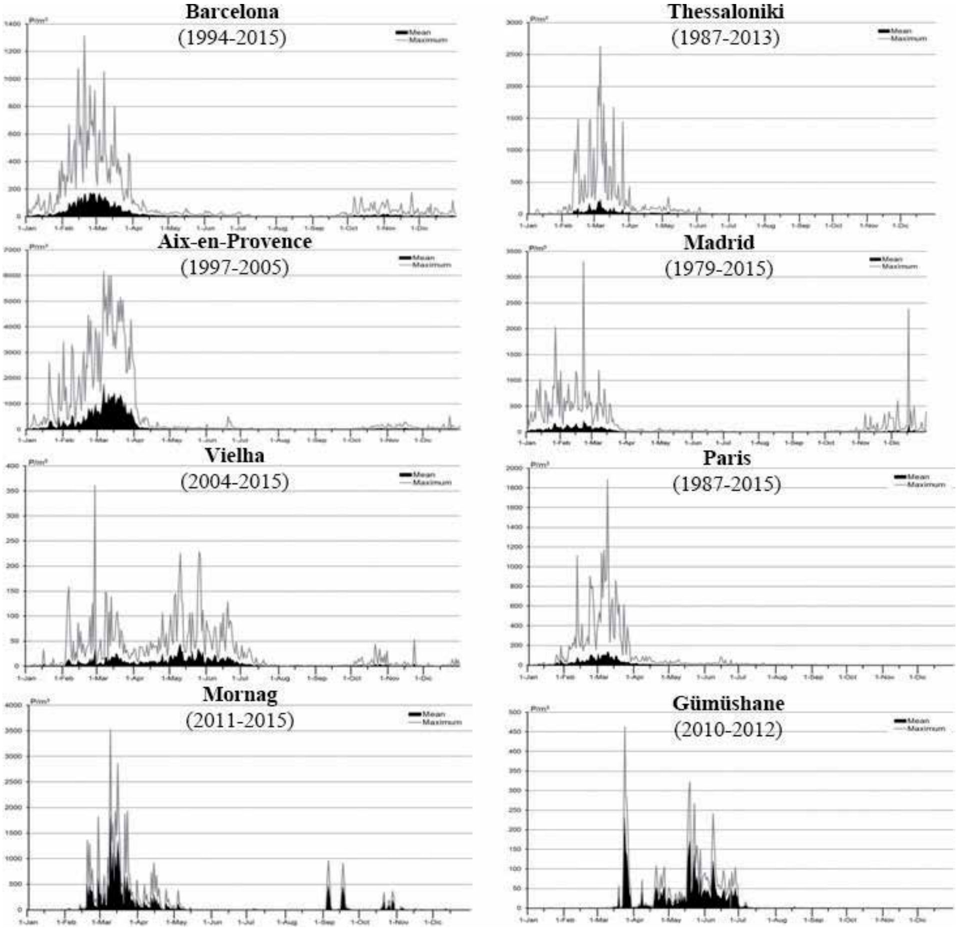

Figure 3.
Cupressaceae pollen dynamics over the course of the year in the Mediterranean area: Barcelona (Spain); Aix-En-Provence (France); Vielha, (Spain); Mornag (Tunisia); Thessaloniki (Greece); Madrid (Spain) and outside Mediterranean area: Paris (France); and Gümüshane (Turkey). Mean daily (thick black line) and maximum daily (thin black line) pollen concentrations are indicated for the period.

Palma de Mallorca, Balearic Islands, Spain [10], and 14% in Nerja, southern Spain [11]. Cupressaceae pollen is also abundant or present outside of the Mediterranean region: Northern Europe, 8% for Cupressaceae and Taxaceae in Munster, Germany [28], South America, 30% in Bahia Blanca, Argentina [29], North America, 18% in Mexico [30], 5–10% on the east coast of the United States [31, 32], and up to 3872 pollen grains/m^3 in January in Tulsa, Oklahoma, central United States where *Juniperus ashei* is predominant [33, 34], Asia, 19% in Yunnan, China [35], and 60% in Japan because of the huge presence of *Cryptomeria japonica* [36], and finally 3% in Santa Cruz de Tenerife, Canary Islands, Spain [37].

2.1.2.2 Aerobiology

Comparative sampling methods developed during the last decades of the twentieth century showed that concentrations of airborne pollen diversity have steadily progressed [36–39].

At least four indices exist to characterize the dynamics and patterns of airborne pollen: *(a):* the mean daily pollen concentration, expressed as the number of pollen grains per cubic meter of air (P/m^3); *(b):* the annual pollen index (API), which corresponds to the sum of the mean daily pollen concentration for each year); *(c):* the dates corresponding to the beginning and end of the pollination; and finally *(d):* the duration of the pollen season.

In the case of cypress pollen, the pollination period (except in mountainous and in very cold sites) begins in autumn and lasts until the end of the following spring. However, in the Mediterranean area, days without any cypress pollen are rare. Therefore a percentage method was proposed. The season is considered to begin when 2.5% of the API has been reached and finished when 97.5% was reached. This method is not totally satisfactory because of substantial year-to-year variations of API.

Using pollen collectors mainly located in urban areas, the Cupressaceae API showed increasing trends in Mediterranean countries. This was shown in Southern France with an early pollination onset [40], in Greece [41] or in Catalonia (NE Iberian Peninsula). Two of these API trends, for Barcelona and Vielha, are shown in **Figure 4** together with the trends in other localities around the Mediterranean. These trends were confirmed for 23 taxa from 13 European countries (97 sites) [42]. Authors did not find any correlation with variation of temperature and rather proposed, as an explanation, the extensive use of Cupressaceae as ornamental plants in the cities. Ariano *et al.* [43] have, however, attributed to climate change a possible role in variations in pollen seasons and allergic sensitizations.

The daily pollen concentration threshold levels required to elicit allergic symptoms in patients remain a crucial question, and no general agreement has been reached. For instance, in Israel, the threshold is considered to be between 10 and 50 pollen grains/m^3, whereas in France, different thresholds of symptom risk have been established for the Mediterranean area (designated as low, when 7–13 pollen grains/m^3, moderate when 14–141 pollen grains/m^3, and high >141 pollen grains/m^3, respectively), and for the north and center of France (designated as low when 70–141 pollen grains/m^3 and moderate when >141 pollen grains/m^3 [44]). The Catalan Network of Aerobiology defined the risk of allergy as being low when concentrations are <20 pollen grains/m^3, moderate for 20–50 pollen grains/m^3, high for 50–100 pollen grains/m^3, and very high when >100 pollen grains/m^3. Furthermore the risk to develop allergy symptoms was shown to be increased by airborne pollutants, especially $PM_{2.5}$ and suspended particulate matter [45].

2.1.2.3 Allergenicity of cypress pollen

The cypress pollen is considered to be highly allergenic (see, for instance, the website of the French National Network of Aerobiological Surveillance, RNSA,"Réseau National de Surveillance Aérobiologique", www.pollens.fr). The allergenic potential of specific pollen depends on the following:

- the degree of exposure, related to the total pollen amount released in the atmosphere (from intact or fragmented grains);
- the phenological conditions in the considered area;
- temperature, hygrometry, photoperiod, …;
- air pollution.

The exposure to cypress pollen is high because of an abundant production of pollen (see pollen chapter), making of this pollen the most represented in the atmosphere (up to 40% of total pollen counts around Marseille in the south of

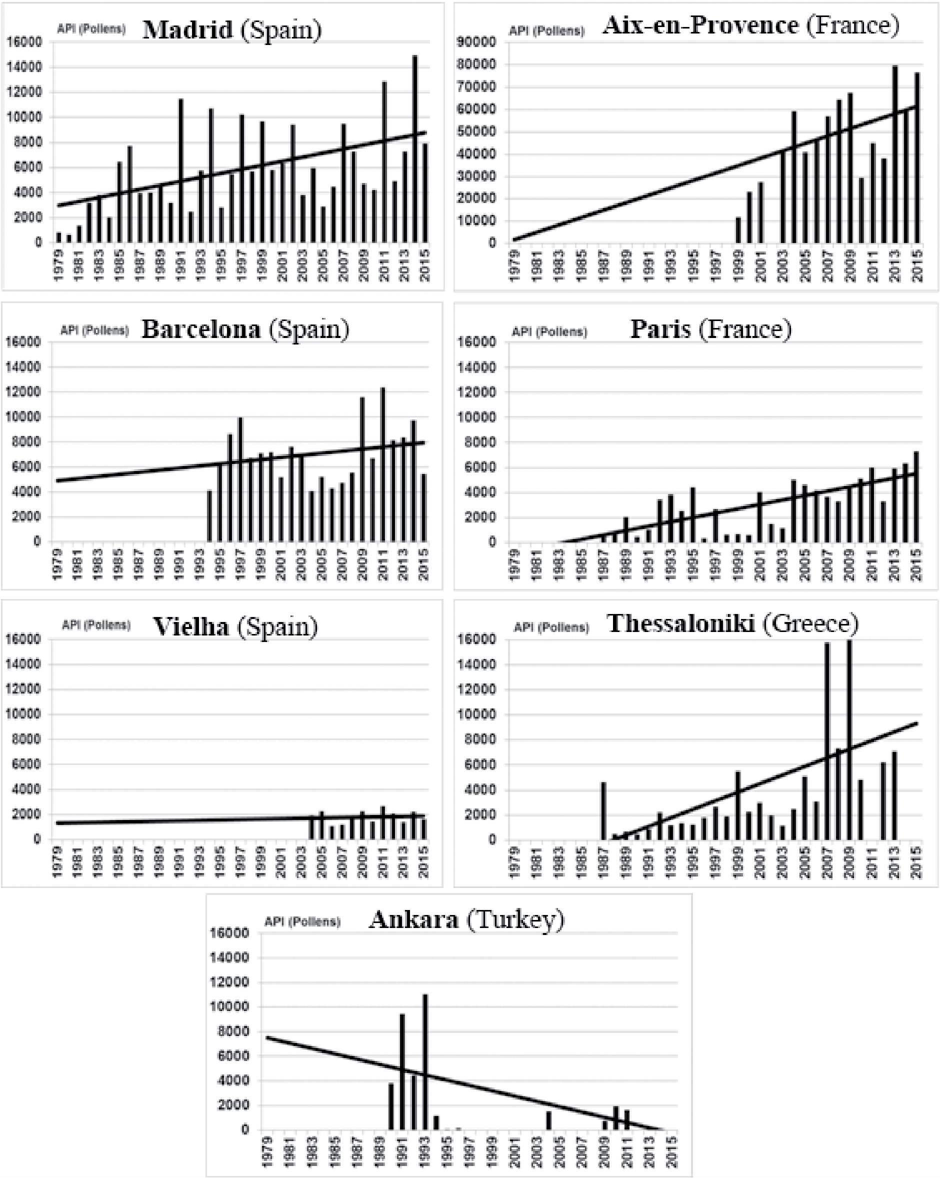

Figure 4.
Cupressaceae annual pollen index (API) and trends at the localities with the longest continuous data series. Madrid (Spain), Barcelona (Spain), Vielha, (Spain), Aix-En-Provence (France), Paris (France), Thessaloniki (Greece), and Ankara (Turkey).

France). Not only is the load high but also the spreading, since rather small pollen, can be wind-transported. Moreover, the pollen grain carries sub-micronic particles named orbicules on its surface (**Figure 2A**). These orbicules were shown to contain allergens from the groups 1 and 2 [46–50] (see below for the definition of groups of allergens) and to be easily released upon rainfall and an experimental *in vitro* treatment of cypress pollen grains with NO_2, a gas frequently found in gaseous pollutants [46, 51]. Because of their small size (300–600 nm), orbicules might be able to penetrate deeper in the bronchial tract and sensitize individuals to induce asthma, as was shown in a rat model [52]. However, free airborne orbicules have never been evaluated; therefore such an orbicule-sensitizing mechanism, in real conditions, was not as yet demonstrated. Besides these characteristics, and in addition to protein allergens, some biogenic intrinsic adjuvant molecules may contribute to the allergenicity of cypress pollen [53–55] by favoring Th2 immune responses and/or triggering innate immune responses. For instance, NADPH oxidase, an enzyme that generates reactive oxygen species, is reported to be involved in inflammation [56, 57]. This enzyme, required for pollen tube growth, is intrinsically expressed at different levels in the pollen grain of various plant species [58, 59]. Cypress pollen is one of the tree pollens containing the highest level of redox activity as compared with other pollen and in particular to the poorly allergenic pine pollen, which expresses the lowest redox activity. Moreover, other biogenic cofactors, pollen-associated lipid mediators (PALMs), play a role in pollen allergenicity. Studies performed on cypress pollen show that lipids of the pollen membrane interact with CD1+ dendritic cells to activate CD1-restricted T cells with the Th0/Th2 phenotype [54]. PALMs are also able to activate eosinophils and neutrophils and decrease IL12 production from dendritic cells, therefore, favoring Th2-biased immune responses [60–64].

2.1.3 Allergens

2.1.3.1 Cypress pollen allergens

C. japonica was the first cypress pollen studied at the level of allergen content, and in 1983 Cry j 1 (previously called SBP, for Sugi Basic Protein) was reported to be its major allergen [65]. Several other allergens were then described in *C. sempervirens* and *Hesperocyparis arizonica* [66–68]. All research groups deciphering allergens from the various cypress species reported a cross-reactive 42–43 kDa protein as being the major cypress allergen: Cry j 1 for *C. japonica*, Cup a 1 for *Hesperocyparis arizonica*, Cup s 1 for *C. sempervirens*, etc... (see **Table 1**). As compared with grass, olive, or ragweed pollen, the extraction of proteins from cypress pollen is difficult, yielding few proteins. This particularity may be related to the high sugar content of the intine quickly swelling when the pollen grain is hydrated; this might delay or prevent the release of proteins [15] (**Figure 2B**). One way to circumvent this difficulty is to grind the pollen in suspension with silica microbeads.

Up to now, five groups of allergens have been described in cypress pollen, although all allergen members for each species have yet to be referenced in the International Union of Immunological Societies (IUIS) allergen data bank (www.allergen.org): group 1: pectase lyase, group 2: polygalacturonase, group 3: thaumatin-like protein, group 4: Ca-binding protein, group 5: Gibberellin-regulated protein. Furthermore, about 20 additional allergens have been reported in the three most studied pollens, *C. japonica*, *Hesperocyparis arizonica*, and *C. sempervirens* (**Table 1**). More details on cypress allergens are reported in Charpin et al. [1] and Poncet et al. [69].

2.1.3.2 Cross-reactivities

- Pollen/pollen

Cross-reactivities between pollen are common because proteins may belong to families of panallergens, such as Ca^{++}-binding proteins or profilins. Some cross-reactivities were observed with *Podocarpus gracilior* of the Pinales order [70], although other authors did not find any cross-reactivity of *C. sempervirens* with pine pollen [71]. *Parietaria judaica*, *Lolium perenne*, and *Olea europaea* pollens were shown to exhibit some degree of cross-reactivity [72] although the nature of the involved allergens remains unknown.

- Pollen/food

Like for pollen from birch, mugwort, grass, ragweed, olive, plane, cypress pollen sensitization was shown to be associated to food allergies. In general, up to 60% of food allergies are associated with an inhalant allergy [73]. A pollen food allergy syndrome (PFAS) has been described, including mainly an oral allergy syndrome. As soon as 2000, Ishida *et al* reported PFAS in patients allergic to Japanese cedar pollen following consumption of specific vegetables and fresh fruits (e.g., melon, apple, peach, and kiwi) [74]. The cypress/peach syndrome was mostly studied [75, 76]. Symptoms might, in some conditions, with cofactors, be more severe than an oral syndrome, up to an anaphylactic shock [77]. An uncharacterized (putative Cup s 1 or Cup s 2) allergen of 45 kDa was proposed to be the cross-reactive allergen [76], and more recently the allergens from the Gibberellin-regulated protein family (group 5 allergens) were shown to be cross-reactive with peach, Japanese apricot, citrus, and pomegranate [77–80]. Prevalence of sensitization to Pru p 7, the GRP from peach, coincides with the prevalence of sensitization to cypress pollen in France [81].

2.2 Epidemiology of cypress pollen allergy

Cypress pollen allergy was reported for the first time in 1929 in the United States (Texas and New Mexico) [82] and in the early 1960s in Europe [83]. Cypress pollinosis is also reported in several locations worldwide: Japan [84], Australia [85], Iran [86], South Africa [87], the United States, and with special emphasis around the Mediterranean basin [88–94] (**Figure 1**).

2.2.1 Prevalence of sensitization and allergy to cypress pollen in non-selected populations

There are consistent correlations between exposure to Cupressaceae/Taxaceae pollen and the presence of sensitization and allergy [95]. Studies performed in the general population are scare. In southern France and in Italy, two studies performed in children [96, 97] and one study in young adults [98] concluded that around 2–4% might suffer from cypress pollen allergy. A study performed in Japan led to a number of *Cryptomeria* pollen allergy of 13% [97–99].

2.2.2 Prevalence of sensitization and allergy to cypress pollen in outpatients

In surveys performed in Mediterranean countries, 14–32% of patients attending an allergy clinic had an allergy to cypress pollen [1]. In a larger Italian study from Rome, 23,077 outpatient sera were studied. The presence of specific IgEs against 75 allergens was investigated, and 42.7% of the subjects exhibited specific IgEs against cypress pollen. In this survey, cypress allergy was the leading cause of sensitization in adults over 35 years of age (in children, house-dust mite allergy was the leading cause) [100]. In Montpellier, a cross-sectional study performed in 400 outpatients concluded that cypress pollen sensitization (20.7%) ranked third, after sensitization to *Dermatophagoides farinae* (37%) and *pteronyssinus* (43%) allergens [101].

2.2.3 Increase in the prevalence of cypress pollen allergy

Several cross-sectional surveys carried out repeatedly over time showed an increase in the proportion of cypress allergy among outpatients consulting for allergic rhinitis: rising from 9.9% in 1991 to 24.5% in 1993, then to 35.4% in 1994 in central Italy [102], from 9.3 to 30.4% between 1994 and 1999 in the area around Rome [103], and from 7.2 to 22.0% between 1995 and 1998 in Italy's Latium area [104]. A recent study in the southern region of Italy showed that cypress pollen sensitization almost doubled from 2005 (17%) to 2010 (29%) [105]. Eighteen were sensitized to cypress or Taxodiaceae pollen in an Italian survey of 3057 outpatients selected in 12 study centers [106]. The sensitization rate was higher in southern Italy (20.1%) and central Italy (28.2%) than in northern Italy (9.2%). In a more recent survey, the geographical hierarchy was unchanged, but the prevalence figures went up to 32.7%, 62.9%, and 16.1%, respectively [107]. A study performed in western Liguria demonstrated an upward trend, whereas pollen counts remained unchanged [108, 109]. This study, like the one by Mari *et al.*, suggests that confounding factors, such as the quality of allergenic extracts, might at least partially explain these discordances [102]. On the other hand, a gradual increase in pollen load, pollen allergenicity [1, 110], and interaction in the patient between air pollutants and cypress allergens [95] are clear indications of a genuine increase.

The rationale for such a rapid increase in prevalence mainly lies with the fact that:

- millions of cypresses were planted in the 1970s and 1980s in the suburbs and around private houses and blocks of flats to offer a degree of privacy. Therefore the proximity of pollen sources has drastically changed: whereas Cupressaceae were traditionally planted in agricultural zones, away from dwellings, nowadays they are planted as hedges to as visual barriers.

- at the same time, a decrease in farming allowed for the extensive proliferation of *Juniperus* in the countryside.

- lastly, in urban areas, air pollution interacts with pollen to increase the allergenicity [51] (see below).

2.2.4 Risk factors for developing cypress pollen allergy

2.2.4.1 Repeated and heavy exposure

In contrast to other respiratory allergic diseases, part of cypress pollen allergic patients have no personal or familial history of allergic diseases, and in this subgroup, the onset of symptoms occurs at an older age [111, 112]. Therefore, even non-atopic individuals repeatedly and heavily exposed during many years to cypress pollens can develop this allergic condition. In high exposure areas, the general population may become allergic to this pollen.

2.2.4.2 Air pollution

Ishizaki et al. first noticed in the 1980s the association between Cupressaceae pollen allergy and air pollutants [84]. They found that living near Japanese cedar trees in urban areas tended to increase the allergy risk compared with living near these trees in rural areas. Concomitantly in Japan, Muranaka *et al.* demonstrated the adjuvant effect of diesel exhaust particles on IgE reactivity to Japanese cedar pollen in mice [113]. The rising prevalence of these pollen allergies observed between 1987 and 1991 (from 17 to 25%) in Japanese mountainous areas could then be partly explained by a drastic increase in the diesel vehicle fleet [114]. Japanese cedar pollen grains in urban areas may adsorb major urban gaseous pollutants such as NO_2, SO_2, and NH_3. Besides, Japanese studies also demonstrated that, on the exine surface, pollutants may be attached, thereby modifying the morphology and ionic composition of pollen grains [115, 116]. This phenomenon could facilitate the release and dispersion of pollen-derived particles smaller than pollen into the atmosphere [46]. During the Japanese cedar pollination period, the level of particle matter ($PM_{2.5}$) and suspended particle matter is therefore increased [117], which induce a negative impacts of this increase on the respiratory health of allergic patients [45].

Since then, Cupressaceae pollen grains have frequently been used as a model to study the interrelationship between air pollutants and pollen allergies [51, 118]. The effects of pollution on the molecular and developmental biology of Cupressaceae pollen has been exemplified by several studies. In polluted areas, the accumulation of numerous inorganic elements such as sulfur, copper, aluminum, and iron on pollen grains and the acidification of pollen surfaces by the adsorption of acid gases such as nitric and citric acids were demonstrated. The natural exposure of Arizona cypress pollen to air pollutants in Barcelona and Madrid promotes the production and release of an allergenic protein (*Cup a* 3) of the pathogenesis-related family 5 (PR-5 protein) [110, 119]. Therefore, the allergenic content of cypress pollen grains could be modified by urban air pollution. Interestingly, levels of adsorption of gaseous pollutants vary greatly, in *in vitro* exposure studies, among different plant species, and cypress pollen seems to be one of the most impacted. The kinetics of NO_2 uptake by cypress pollen is two and six times that of grass and birch pollen, respectively [120]. Furthermore, allergen-carrying free orbicules are generated following exposure of cypress pollen to NO_2 [51]. More comprehensive and experimentally designed studies on the interrelationship between pollen, air pollution, and respiratory allergies should derive from these recent physicochemical experiments.

2.3 Clinical and management aspects

2.3.1 Symptoms and diagnosis

According to the Japanese survey [99], and a subsequent study from Europe, rhinitis is more common than conjunctivitis. The latter is, however, the most disabling symptom, occurring in 72% of patients allergic to cypress pollen, versus 26% of patients allergic to grass pollen [111]. In this study, the occurrence of a chronic cough was much more frequent with cypress pollen allergy, whereas asthma symptoms during the pollen season were equally prevalent in patients allergic to grass and cypress pollens. Besides, allergy to cypress pollen was more disabling than other pollen allergies, according to a visual analog scale used by 4025 patients visiting their general practitioner for allergic rhinitis [121].

The diagnosis of cypress pollen allergy mostly relies on the clinical history, which is usually highly suggestive because most cypresses pollinate in wintertime when no other airborne pollens are present. The diagnosis is supported by skin tests, using either a mixture of *C. sempervirens* and *Hesperocyparis arizonica* or extracts from *J. ashei*. However, in a few cases, despite the convincing medical history, skin tests are negative. [122]. The allergist can ask for specific IgE measurement. Measurement of specific IgE to *J. ashei* has proven to be more sensitive than IgE directed toward *Cupressus* allergens [123]. In few cases, the patient is indeed sensitized to cypress pollen, but the relevance of this sensitization in the clinical picture is questionable. Then, measurement of serum recombinant Cup a 1, which evaluates antibodies directed to the major allergen, can be useful [122].

2.3.2 Management of cypress allergic patients

2.3.2.1 Pharmacological treatment

Compared with other allergic diseases, no specific pharmacologic treatments are given for this condition.

2.3.2.2 Immunotherapy

Although they only included a limited number of patients, several clinical trials have addressed this issue [1]. A benefit in terms of symptoms, quality of life, on-demand medications, late cutaneous response to allergen, and specific nasal hyperactivity was demonstrated in all trials. Clearly, larger clinical trials including longer treatments and longer follow-up periods are required.

2.3.2.3 Individual avoidance procedures

While all of these procedures are based on common sense, they have not been clinically validated [124]. This paper demonstrated that four recommendations are provided by most scientific committees and organizations: avoiding outdoor activities, consulting pollen forecasts, avoiding drying laundry outdoors, and wearing pollen protective glasses and mask when outdoors. All these pieces of advice are applicable when the taxon to which an individual is sensitized is present.

2.3.2.4 Collective strategies

Integrated strategies have to be developed to prevent cypress pollen allergy, in addition to medical care and desensitization. The reduction in individual exposure to pollen is the upstream component of this strategy. Pollinosis is more frequent in urban areas, although airborne pollen concentrations should be lower than in rural areas [125]. Therefore, the allergenic features of ornamental plants that are used in urban green spaces, parks, and gardens should be taken into account in future urban planning [126]. Should be chosen over allergenic wind-pollinated species non-allergenic species and/or insect-pollinated species the use of the latter species should be reduced in order not to aggravate their impact on allergy sufferers, even if cultural and historical reasons often make this a difficult choice.

A "Database of Urban Tree Potential Allergenic Values," integrating the different components of the allergenicity risk (e.g., tree size, type of pollen dispersal type, flowering period, etc.), has been generated for all of the individual trees producing an estimate of the allergenicity of Urban Green Zones [127, 128].

People with pollen allergies could limit their exposure to pollen through consulting forecast of pollen emissions based on phenological modeling of pollination. They should avoid spending time in areas with high densities of Cupressaceae taxa. Because pollen penetration in summer was estimated to be one hundred times higher than in winter and although pollen is much more abundant in winter, the penetration of pollen into dwellings must also be minimized by avoiding the opening of doors and windows in the summer time [129]. This is all the more true that cypress pollen allergenic potency was shown to last over at least a 10-month period in an indoor environment [130].

Trimming of isolated trees or hedges before pollination represent a complementary strategy to reduce the amount of pollen produced by Cupressaceae trees. It can significantly reduce pollen production [131]. An efficient medium- to long-term way to reduce atmospheric pollen loads without the need to eradicate the Cupressaceae species in urban areas could be to select low pollen producing varieties. Female cultivars are preferable for the few monoecious species. Low pollen cultivars should be selected for other species, either in natural populations or breeding populations, as for *C. japonica* [132]. For this latter species, an approach to prevent pollen dispersal lies in the use of pollen-specific fungal infection [133]. For *Cupressus*, sterile cultivars can be produced through the production of haploid lines from *C. dupreziana* surrogate mothers [134].

3. Pinaceae

As stated in the introduction, Cupressaceae/Taxaceae and Pinaceae are the three families of conifers studied at an allergy point of view. Pinaceae is mentioned as poorly allergenic in the RNSA data bank despite a huge amount of pollen produced. Eleven genera were described distributed in four subfamilies and 220–240 species. Two genera are presented below, *Pinus* and *Abies*.

3.1 Pinus

From the family Pinaceae, the genus *Pinus* includes about 120 species. The main species studied at an allergy point of view are *Pinus pinea, halepensis, radiata, sylvestris,* and *nigra.*

3.1.1 Trees and pollen

Pine trees are evergreen, conifer trees with leaves as needles bundled in clusters called fascicles. Pines are mostly with male and female cones on the same tree. The male cones are mainly present in spring, falling after pollen shedding. The female cones have numerous spirally arranged scales, with two seeds per scale. Some pine seeds (pine nuts) are edible and have been reported to induce allergies. Pine pollen grains are 40–80 μm diameter, are heavy, and harbor a waxy hydrophobic coat. They are easily distinguishable under microscope observation because of two balloons filled with air. This particularity does not help the pollen to float in the air but rather to float on a water surface. The tree is anemophilous, and pollination is abundant generating the so-called "sulfur rain" during pollinating season [135, 136].

3.1.2 Allergenicity

Despite the sometimes widespread pine forest and the abundance of pollen grains, the allergenicity of pine pollen was considered very poor if not nonexistent by some authors [137–144]. The involvement of pine pollen in seasonal allergic reactions has been evaluated in some studies and has generally been considered of little clinical significance. For example, Harris and German, in 1985, evaluated 200 patients during the pine pollen season [145]. Among them, only five had a positive skin test to pine pollen (*Pinus radiata*), i.e., about 2%. Kalliel and Settipane reported 6% using *Pinus strobus* pollen [146], and Armentia *et al* described three cases with *P. pinea* including one patient also sensitized to pine nuts [147]. Cross-reactions were reported with ray-grass, but some genuine sensitization to pine pollen could also be demonstrated in *P. radiata* [71, 148, 149]. In another study involving 826 patients in northern Arizona [150], only 12 (1.5%) had a positive skin test to pine pollen (*Pinus ponderosa*). Among them, eight reported a rhino-conjunctivitis during the pollen season while four had perennial symptoms. However, a paper originating from an area with high exposure to pine trees (north-west of Spain) described a series of 10 patients sensitized to pine pollen (*Pinus pinaster* and *radiata*) with symptoms during the pine-pollen season, among whom eight were mono-sensitized [151]. As well, in Canada, an increase in pollen from Pinaceae (pine, fir, spruce), Tsuga (hemlock), and Larix (larch, tamarack) was shown to play a role in increase of daily hospitalization for asthma [152]. These studies did not result in the description of specific pine pollen allergens. Allergens were only reported in pine nuts and correspond to storage proteins, 7S vicilin, 2S albumin, and a 17 kDa protein [153–155].

3.1.3 Hypotheses for low allergenicity

There are several hypotheses to account for this low apparent clinical significance, which is at variance with the heavy pollen exposure in areas densely covered with pines. Firstly, there might be an underestimation of the sensitization rate because protein extraction from pine pollen is difficult [156]. In comparison to classical extraction protocols such as soft incubation in aqueous solution, grinding of the pollen grains together with 1 mm silica beads results in 20–50 times more extracted proteins amount (**Figure 5**) [157]. The improvement of the extraction is not only quantitative but also qualitative. Interestingly Pasaribu *et al*, using adapted extractions protocols showed sequence homologies between oleosins from pine nuts and pine pollen [158]. Oleosins have been reported to be allergenic in sesame, peanut, and hazelnut, but classical protocols do not allow the extractions of these hydrophobic proteins. Secondly, the pine pollen might have a low allergenic potency because intrinsic compounds, which have been shown to play a role in enhancing a Th2 immune response via innate immunity, are deficient. For instance, the enzyme NADPH oxidase, proteases, and PALM (pollen-associated lipid mediators) contents are low in pine pollen [56, 159, 160]. NADPH oxidase leads to generation of reactive oxygen species, and PALM boosts Th2-type allergic reactions [161]. Finally, similarly to other airborne allergenic sources, pollution and climatic change have an impact on the allergenicity of pollen grain, and allergenicity of pine pollen was indeed shown to be affected by O_3 [162].

3.2 Abies

3.2.1 Trees and pollen

From the family Pinaceae, the genus *Abies* includes 46 species. They originated from temperate and north hemisphere, and fir is the most represented. They are

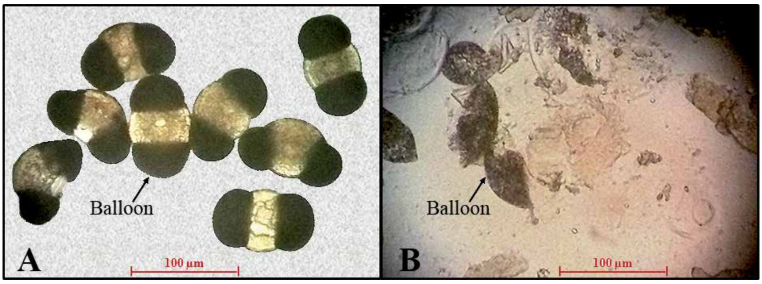

Figure 5.
*Pine pollen (*Pinus halepensis*) observed under optical light microscopy, (200x magnification) from Brazdova et al. [157]. A: Intact pollen grains. B: Grinded pollen using a multidirectional grinder (fast-prep 24-5G, cool prep, MPBiomedicals) in the presence of 1 mm silica beads. The disruption of pollen grains results in qualitative and quantitative enriched protein extraction.*

found in North and Central America, Europe, Asia, and North Africa, occurring mostly in mountains. They are large trees, reaching heights of 10–80 m tall when mature. Firs can be distinguished from other members of the pine family by the way in which their needle-like leaves are attached singly to the branches with a base resembling a suction cup and by their cones, which stand upright on the branches like candles and disintegrate at maturity. The leaves are significantly flattened with an upper surface uniformly green and shiny. Fir trees produce very large amounts of pollen annually in the spring and early summer. The pollen grains are large (160 μm) and similar to the pine pollen grains exhibiting two balloons filled with air.

3.2.2 Allergenicity

Abies pollen is considered barely allergenic, and only one study mentioning fir pollen together with other Pinaceae pollen has been carried out so far in Canada (see above [152]). No prevalence is reported and no allergens are described.

Fir is present in many homes during Christmas time, and there are a few reports of rhinitis and conjunctivitis occurring during and following Christmas tree exposure [163]. However, authors concluded that these symptoms were not pollen-dependent but rather caused by volatile organic compounds emitted by the tree since fir pollen grains have disappeared at Christmas time. One of these compounds was identified as colophonium shown to be able to sensitize allergic patient to induce dermatitis [164]. Another confounding and misleading factor could be mold spores contaminating the Christmas tree [165]. Mold spores such as *Aspergillus* or *Cladosporium* are well-known allergenic sources.

4. Conclusion

Out of the seven families described in conifers, obviously the Cupressaceae/Taxaceae was the most studied precisely because its wide distribution and the powerful allergenic potential of its pollen giving rise to a high prevalence where Cupressaceae/Taxaceae is implanted. Furthermore numerous associations with food allergy were reported inducing not only respiratory but also food allergy symptoms from the oral syndrome to more severe outputs such as systemic anaphylaxis or urticaria. Sensitization can occur lately, in non-atopic individuals and thus, represents a public health threat. However, compared with ragweed or grass pollen allergy, Cupressaceae trees rarely spontaneously reproduce, and their expansion could then be controlled by policymakers.

Pinaceae pollen allergy was also studied, though to a lower extent, because pollination is huge despite a very low prevalence. Therefore it does not represent an important health issue. The question of food cross-reactivity was also addressed, especially with the edible pine seeds, but no convincing data were published.

However, the climatic change and polluted environment might result in a general trend to increase allergenicity of airborne allergenic sources, including pollen. Therefore an immunosurveillance and health monitoring should be maintained for all pollen species.

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Conflict of interest

The authors declare that they have no conflict of interest concerning this article.

Author details

Denis Charpin[1]*, Hélène Sénéchal[2] and Pascal Poncet[3]

1 French Clean Air Association, Aix-Marseille University, Marseille, France

2 Armand Trousseau Children Hospital, APHP, Paris, France

3 Immunology Department and Armand Trousseau Children Hospital, APHP, Institut Pasteur, Paris, France

*Address all correspondence to: charpindenis27@gmail.com

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Conifers include a wide range of species that are spread all over the world. These species have wide diversity, variable stand structures ranging from monospecific monocohort to multispecific multicohort, and produce an assortment of products and services, the most frequent of which is timber. This book is a collection of contributions, both reviews and research studies, from different fields and perspectives on the management, regeneration, growth, diversity, and production of conifer stands. The book also addresses the effect of wildfires on conifer ecosystems and respiratory allergies to conifers.

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