UNIVERSITÀ DEGLI STUDI "MEDITERRANEA" DI REGGIO CALABRIA
DIPARTIMENTO DI AGRARIA
Dottorato di Ricerca in
Scienze Agrarie, Alimentari e Forestali
Curriculum Scienze Forestali
Ciclo XXXV, 2019/23 - SSD: AGR/05

Michele Mercuri

Can the presence of *Heterobasidion* spp. affect the productivity of beech-silver fir pure and mixed forests?

PH.D. THESIS

Tutor
Prof. Fabio Lombardi

Co-Tutor
Prof. Nicola La Porta

Ph.D. Coordinator
Prof. Leonardo Schena

Reggio Calabria 2023

Index

Abstract

The aim of this Ph.D. project is to investigate whether the presence of pathogenic conifer root rot fungi, such as *Heterobasidion* spp., can influence the productivity, the vitality and the growth trends of beech and silver fir forests, but also the wood quality of the materials harvested from them. Specifically, the research work is based on the hypothesis that mixed forests of beech and silver fir (*Abies alba* Mill. - *Fagus sylvatica* L.) may be better protected from root rot pathogens than the pure silver fir stands, and in particular from *Heterobasidion* spp., which mainly causes damage to conifers. The study was conducted in the context of the “Serre” Regional Park (Calabria Region, Southern Italy), specifically within two Special Areas of Conservation of the Natura 2000 Network. The work was conducted on different study in chapters 3 and 4 that have a common denominator that involve species studied in similar bio-geographical settings. A in-depth bibliographic research was carried out, with the aim of drawing up a review of the state of the art regarding the relationship between the presence of *Heterobasidion* spp. and the tree growth dynamics and tree vitality. By means of dendrometric analyses and dendrochronological approaches, it was tested whether the mixing of silver fir and beech species could improve the growth and fitness of silver fir, when compared to pure silver fir stands, hypothesizing that the presence and propagation of roots infected with *Heterobasidion* spp. could be limited by the mixing of species, thus reducing its propagation through trees. Lastly, an attempt was made to verify whether the quality of the woody products and their biomechanical characteristics can be improved by promoting the mixture between tree species, with particular reference to pure and mixed forests of beech and silver fir. In order to test this hypothesis, non-destructive methods (TreeSonic timer) were used to estimate the Dynamic Modulus of Elasticity (MOEd) and infrared spectroscopy (TF-IR spectrometer) was also used to estimate the presence and abundance of molecular components such as lignin and cellulose. For this purpose, an LDA+clustering model was also applied. In addition, a model based on linear discriminant analysis was constructed and developed in Python to distinguish different forest types on the basis of the IR spectrum. It was found that silver fir had a higher hypso-diameter

ratio and higher tree density when growing in pure forests, while fewer and larger individuals were observed in the mixed stands . On the basis of the results obtained, the presence of the pathogen *Heterobasidion* spp., and in particular the intersterile group F (*H. abietinum* Niemelä & Korhonen) was ascertained; it was found that the annual increments variations are lower in the pure stands than in the mixed stands for silver fir. Furthermore, the mixed stands ensured a greater increase for silver fir volume than in the pure stands, thus demonstrating that the mixed forests contributes to a more stable system, which also ensures greater productivity. In addition, the structural and dendrochronological analyses developed in chapter four showed more regular growth trends in the mixed stands than in the pure ones, and the benefits on wood quality were confirmed by the higher MOEd values observed in the mixed plots. Furthermore, higher levels of cellulose and lignin, detected by TF-IR spectroscopy, were observed in the silver fir-beech mixed stands. Therefore, the results obtained suggested that the promotion of mixture of tree species in mountainous forest ecosystems could improve the tree growth dynamics and wood quality of beech more than silver fir, together with the reduction of root and butt decay. The better wood quality observed in the mixed forests, probably due to the effects above mentioned, is further confirmed by the values obtained for the modulus of elasticity, which were higher in the mixed forests, indicating a higher structural homogeneity of the woody material.

Riassunto

Il progetto di dottorato si è proposto di verificare se la presenza di funghi patogeni di marciume radicale delle conifere, come *Heterobasidion* spp., possa influenzare la produttività di boschi di faggio e abete bianco e la qualità del materiale legnoso da essi ritraibile. Nello specifico, il lavoro di ricerca si è basato sull'ipotesi che i boschi misti di faggio e abete bianco (*Abies alba* Mill. - *Fagus sylvatica* L.) possano essere maggiormente protetti dai patogeni radicali, ed in particolare dall'*Heterobasidion* spp. che arreca danni principalmente alle conifere, rispetto a boschi di conifere puri a dominanza di abete bianco. Le attività sono state condotte nel contesto forestale del Parco Regionale delle "Serre" (Calabria), nello specifico all'interno di due Zone Speciali di Conservazione della Rete Natura 2000. Il lavoro è stato condotto su diverse aree di studio nei capitoli 3 e 4, che hanno un denominatore comune: le specie studiate in contesti bio-geografici simili. E' stata dapprima realizzata un'approfondita attività di ricerca bibliografica, con l'obiettivo di redigere una review sullo stato dell'arte riguardante la relazione tra la presenza di *Heterobasidion* spp. e le dinamiche di crescita del bosco. Attraverso analisi dendrometriche e approcci dendrocronologici, si è voluto verificare se la mescolanza di specie di abete bianco e faggio possa migliorare la crescita e la vitalità dell'abete bianco rispetto al soprassuolo puro, ipotizzando che la presenza e la propagazione di radici infette da *Heterobasidion* spp. possano essere limitate dalla mescolanza di specie, riducendo così la propagazione del patogeno tra gli individui arborei. Inoltre, si è cercato di comprendere se la qualità dei prodotti legnosi e le loro caratteristiche biomeccaniche possano essere migliorate promuovendo la mescolanza tra le specie arboree, con particolare riferimento a boschi puri e misti di faggio e abete bianco, Per verificare questa ipotesi, sono stati utilizzati metodi non distruttivi (timer TreeSonic) utili per stimare il Modulo Dinamico di Elasticità (MOEd) e la spettroscopia infrarossa (spettrometro TF-IR) utile per quantificare la presenza e l'abbondanza di componenti strutturali del legno, come lignina e cellulosa. A tal fine, è stato applicato anche un modello LDA+clustering. Inoltre, è stato costruito un modello basato sull'analisi discriminante lineare, sviluppato in Python, per distinguere le differenti tipologie forestali oggetto di studio sulla base dello spettro IR. E’ emerso che le piante di abete bianco presentano un rapporto ipso-diametro più elevato ed una maggiore densità nei boschi monospecifici, mentre nei popolamenti misti è stato riscontrato un minor numero di individui, ma di maggiori dimensioni. E’ stata quindi accertata la presenza del patogeno *Heterobasidion* spp., in particolare del gruppo intersterile F (*H. abietinum* Niemelä & Korhonen) e, in base ai risultati ottenuti, è emerso che gli incrementi annuali sono inferiori nel soprassuolo puro rispetto a quello misto. Inoltre, il soprassuolo misto garantisce un maggiore incremento volumetrico per l’abete rispetto al soprassuolo puro per la stessa specie, dimostrando così che il bosco misto contribuisce a un sistema più stabile, che garantisce anche una maggiore produttività. Inoltre, le analisi strutturali e dendrocronologiche dettagliate nel quarto capitolo hanno evidenziato una crescita più regolare nelle situazioni miste rispetto alle foreste pure, e i benefici sulla qualità del legno sono stati confermati dai valori di MOEd più elevati nelle parcelle miste, insieme ai livelli più elevati di cellulosa e lignina nel faggio misto all'abete bianco rilevati dalle analisi spettroscopiche TF-IR. I risultati ottenuti nel presente lavoro suggeriscono quanto la promozione della mescolanza tra specie in ambienti montani può migliorare le dinamiche di crescita e la qualità del legno del faggio più che dell'abete bianco, oltre a ridurre danni da marciume radicale e del fusto. La migliore qualità del legno in condizioni di bosco misto, probabilmente dovuta agli effetti sopra menzionati, è ulteriormente confermata dai valori ottenuti nella stima del modulo di elasticità, significativamente più elevati nel bosco misto, indicando quindi una maggiore omogeneità strutturale del legno analizzato.

Keywords: *Abies alba* Mill., Serre Regional Natural Park, Tree species mixture, Pathogenic fungi, Tree growth patterns, Dendrocronology, Wood-quality.

CHAPTER 1

1. General Introduction

This research project aims to investigate whether the presence of conifer root rot pathogenic fungi, such as *Heterobasidion* spp. can influence the productivity of beech and fir forests. Specifically, the work is based on the hypothesis that mixed silver fir and beech (*Abies alba* Mill. - *Fagus sylvatica* L.) forests may be better protected from root pathogens, and in particular from *Heterobasidion* spp. which attacks conifers exclusively, than pure coniferous forests dominated by silver fir. In detail, over the course of these years of research, the collaboration with the Edmund Mach Foundation of San Michele all'Adige (TN) has been fundamental, which has allowed the structuring of the protocol for the identification of forest areas affected by the presence of *Heterobasidion* spp., the surveys and the subsequent characterization of the above-mentioned pathogen. The study area fall entirely within the "Serre" Regional Natural Park (Calabria Region) and, more specifically, in two Special Areas of Conservation (SACs afferent to Natura 2000 Network). Two forest types were identified: a mixed forest of *Abies alba* Mill. - *Fagus sylvatica* L. within the SAC "Bosco Archiforo" and a pure forest dominated by *Abies alba* Mill., into the area of the SAC "Bosco Santa Maria," both in the municipality of Serra San Bruno (VV, Calabria Region) where the altitude ranges between 900 and 1,200 m above sea level. During the Ph.D., an attempt was made to verify whether areas with a mixture of silver fir and beech-dominated species (*Abies alba* Mill. - *Fagus sylvatica* L.) are more protected from root transmission by *Heterobasidion* spp. than single-species forests dominated by silver fir, probably because of the lower frequency of root anastomoses. In addition, the quality of the woody products and their biomechanical characteristics were investigated in order to verify if they could be improved by the promotion of the mixture between beech and silver fir. This hypothesis was verified applying non-destructive methods (TreeSonic timer), useful for the estimation of the Dynamic Modulus of Elasticity (MOEd), and infrared spectroscopy (TF-IR spectrometer), useful for the estimation of the presence and abundance of molecular components such as lignin and cellulose. The expected results could be useful to select the most appropriate management practices useful to improve the productivity of the forest ecosystems here investigated. Hopefully, the result obtained will be useful for forest managers to identify the best silvicultural practices aimed to improve the forest productive functions.

1.1. Mixed beech- silver fir and pure silver fir forests

Silver fir is a species that easily tends to the natural formation of mixed forests: in the “Serre” Regional Park, silver fir has presumably found its only real "refuge area" in the Southern Apennines. In this geographic area, beech associates with silver fir forms a highly stable forest stands, capable of ensuring natural regeneration, constant forest floor cover and perfect dynamic balance with the local environment. The mixed forest better performs the function of preserving the natural factors of production and biological functionality by ensuring the perpetuity of the forest. The physiognomy of the mixed silver fir-beech forest has been highly altered over time due to the irrational use of silver fir and the abuse of grazing in forests. Currently, the composition of the mixed silver fir-beech forest is highly variable. Areas where there are very high percentages of one species with discontinuous presence of the other correspond to areas where both silver fir and beech may constitute groups in their pure state (Figure 1).

Figure 1: *Mixed beech-silver fir and pure silver fir forests.*

The tree distribution in the mixed stands often reveals, in a long term perspective, an alternating regeneration of beech under silver fir cover and vice versa. Silver fir regeneration, within these mixed forests, is well supported or, even intrusive, especially if the lower storey consists of broadleaf trees (Magini, 1967). Such regeneration is favored in more acidophilic forest types (Di Tommaso, 1976;

Drapier, 1985), such as Iuzula nivea (*Luzuna nivea*) abieti-fagetum, while in more mesophilic types the undergrowth is more developed and more susceptible to the invasion by tall nitrophilic grasses, although exceptions occur in Calabrian mountains (Ferrarini & Padula, 1969). Thus, silver fir renews well on mineral soils and without difficulty if associated with beech; in fact, their alternation helps to maintain the balance of mountain coniferous forests. If silver fir is mixed with beech, it succeeds in conserving itself as long as the deciduous tree is less competitive. It readily colonizes coppice or disturbed chestnut, but also turkey oak forests and pine forests; however, it fails to renew easily in pure silver fir forests. Ungulate fauna also contributes to hindering the silver fir regeneration, e.g., deer, fallow deer and roe deer, which are attracted by the palatable young juveniles of silver fir, devoting themselves to silver fir and pine juveniles only as rescue food.

In fact, it is believed that one of the causes of the decline of silver fir is precisely the intervention of large grazing herbivores, which go to hinder its establishment in mountain areas, even those derived from reforestation (Bernetti, 1995). "The history of the mountain peopling of the Apennines during the post-glacial period, *sensu stricto*, is the history of the struggle between two climaxes: the Abietum and the Fagetum, the latter of which is absolutely prevalent today over the former". This is the conclusion reached by (1936) on the basis of many researches dealing with the evolution of Alpine and Apennine vegetation in the postglacial period. In this general context, the forests of the “Serre Vibonesi” in Calabria represent particular and characteristic forest types where a balance between silver fir and beech is evident in stable and productive mixed stands. However, the past forest management based on the application of classical forms of treatment referring mainly to the “Successive Cut System”, makes one species prevailing over the other for decades. Silver fir and beech have always constituted important elements in the economy of the local populations of the inner areas. The favorable ecological conditions of this Apennine site, judged by Chiarugi himself (1936) to be similar to those that had favored the prevalence of silver fir, allow the establishment and maintenance of mixed stands. The silver fir is less and less frequent in our forests. On the one hand, for economic reasons, it is being replaced by beech, a more economically attractive species. On the other hand, silver fir appear at the top of the list of favorite foods of deer and roe deer. On fertile soils in our regions, silver fir can reach 50-55 meters in height and live up to 600 years. During this time, they are able to produce more timber than beech, if correctly managed.

Marziliano et al. (2011) focused on the growth patterns of silver fir as a function of the presence-absence of consociation with beech, evaluating the volume increments (current and percentage), growth patterns through dendrochronological approaches and exploring the passage time, as they have always been fundamental elements for the study of forest stand productivity and directly related to the structural elements that characterize the stand. In this context, within the “Serre” Regional Park, it has been of the utmost interest to analyse the relationships occurring between different forest types, in particular pure stands of silver fir of natural origin and mixed stands of silver fir and beech. Interest was directed to the analysis of volume increments (current and percentage increment) and tree growth trends, as these have always been fundamental elements in the study of forest stand productivity and can be directly linked to the structural elements that characterize the stand. It is interesting the comparison between tree growing in stands with different structural traits, particularly pure silver fir forests and mixed fir-beech forests: they can reveal different growth trends, in terms of tree diameter and tree height. In addition, trees grown in the mixed forest exhibit greater diametric increments. Silver fir can reach sizes exceeding 130 cm in diameter, with heights of more than 40 meters (Marziliano et al., 2011). The promotion of the coexistence of multiple forest species, in montane and Mediterranean forests, has recently been emphasized, through studies in national and international contexts (Bravo-Oviedo et al., 2018; Coll et al., 2018; Huuskonen et al., 2021). Research has shown the greater effectiveness of mixed forests in improving resistance and resilience of forest ecosystems to natural disturbances, compared to the corresponding monospecific stands (Forrester et al., 2017; Jactel et al., 2017). Both the concepts of resistance and resilience have been addressed and defined in many studies. In general, the concept of resistance is defined as the ability of a system to absorb an external disturbance with its subsequent rebalancing as a result of being disturbed, while the concept of resilience indicates the ability by a system to respond to biotic agents. Thus, in forest ecosystems, the resilience can be understood as the response and subsequent adaptation or recovery to periodic stresses, such as periods of drought or disturbances that may be induced by fires or windstorms. Resilience, on the other hand, as the ability to cope with attacks from biotic factors, which may be pathogenic fungi or insects; resilience increases in mixed forests, which generally have a reduced presence of pests and, compared to species-pure forests, suffer less damage (Coll et al., 2018). In fact, a species-rich forest can reduce the damage from pathogen attacks, through a reduction in density, from which results in less colonization by fungi (Field, Petrokofsky, e Koricheva s.d.) in addition to the fact that the presence of more species within a forest ecosystem can present itself as a solution to secondary infections by fungi that propagate through root anastomoses, since the presence of plants that are immune to pathogen attack can act as a barrier to the fungi advancement. This particular topic is the focus of the present research study. In addition, the greater specific complexity of mixed forests can positively influence the quality of the woody material produced and, consequently, the final products obtainable in the forest-wood supply chain (Russo et al., 2019; Zeller et al., 2017). Confirming the latter thesis, Russo et al. (2019) demonstrated, in a study of pure and mixed beech and Calabrian pine forests, how the effect of species mixing affected both wood quality and tree ring widths. In both pure and mixed species stands, the values of MOEd (dynamic modulus of elasticity), which is closely related to wood quality, were above the minimum quality threshold (MOEd > 9000 MPa).

Pure silver fir forests are classic formations that develop more on the mountain belt and are found to a greater extent in the Alps, more rarely in the Apennines, typically in endalpic and intermediate positions where silver fir consociates with Norway spruce and other conifers such as larch and Scots pine. Favoring the presence of these consociations are anthropogenic actions and pioneering phenomena by the species; the absence of beech is caused by too continental climatic footprint in these climatic areas. With the decrease in cutting regimes, silver fir has regained its importance, especially in forest areas characterized by acid soils, although excessive acidity makes its regeneration slow. Special formations are found in the Western Alps, such as subalpine fir stands with Alpenrose (*Rhododendron ferrugineum*), which consist of stands that reach an altitude of 1900 m. a.s.l., mixed with larch or, in some cases, with stone pine. In these stands, the development of silver fir appears to be very modest; these are relict formations that confirm the expansion and spread of silver fir at altitudes that occurred in the past. Silver fir can often be found in the basal belt, especially in the Alps, as a consequence of descent to the bottom of wet valleys or to gorge stations, while in the rest of the Italian Peninsula this descent is much more frequent and can be attributed to descent into chestnut or turkey oak coppices, or to relict stations when found in anomalous positions (Patrone, 1952). On the other hand, as far as man-made silver fir forests are concerned, it is very difficult to trace which original genesis type they replaced, especially if planted in fairly remote times. The replacement of maple and beech and other broadleaf forests occurred in sites close to the Apennine ridge where silver fir has a very modest development and has numerous crashes, while that of maple-ash forests occurred in gully or poorly sunny stations, which favor an undergrowth rich in large broadleaf trees such as sycamore maple, greater ash. The silver fir plantations created in sites where chestnut or turkey oak were present exhibit all the negative characters caused by descent to lower altitudes with early senescence and pathogen/parasitic attacks.

In the pure silver fir forests occurring in the “Serre” Regional Park, where the present thesis work was focused, silver fir regeneration is frequent (Ciancio et al., 1985; Kramer, 1984; Mercurio & Spampinato, 2006; Pavari, 1914; Pizzigallo, 1941; Volpini, 1961), and these are either of artificial origin, as in the “Santa Maria” forest (located in the municipality of Serra San Bruno), or stands in which the prevalence of silver fir over beech is the result of anthropic interventions implemented in the past (high-intensity cutting within stands with a predominance of beech) that have favored, above all, the juvenile silver fir. This prevalence, as can be well observed in the same SCI area of “Bosco di Stilo”-“Bosco Archiforo”, is temporary and, at the next use, the present silver fir stand will turn into a practically pure beech forest. The density of the stand limits the presence of the undergrowth, which consists mainly of miscellaneous grasses concentrated mainly in the area where the forest cover is lower, with brambles and sporadic plants of butcher's broom and *Daphne laureola* and others.

1.2. The “Serre Regional Park” as context of the Ph.D. study

The “Serre” Regional Park is the first Regional Park in Calabria, established by Regional Law No. 48 of 05/05/1990 and defined in its boundaries by the Decree of the President of the Region of Calabria No. 138 of 16/12/2003 (Figure 2).

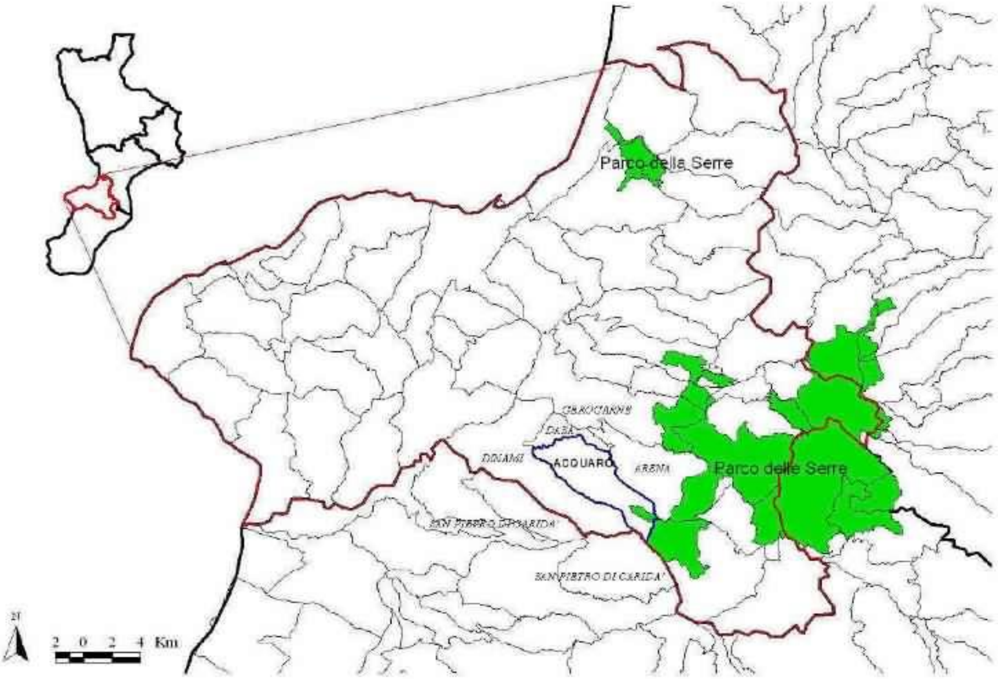


Figure 2: *Location of the “Serre” Regional Park in Calabria Region.*

This area, together with the other resources present in the Region, is subject to considerable touristic attraction, suitable to activate in a systemic action economic and cultural processes capable of revitalizing the territory of the inner areas. The landscape is unique and fascinating, among streams and granite masses, forest cover is large, consisting of majestic silver firs and imposing beech trees. It constitutes one of the least known but also most beautiful mountain groups in the entire Mediterranean basin. The protected area, whose extension is 17,687 ha, unites the two State Nature Reserves Cropani-Micone (237 ha) and Marchesale (1,257 ha), as well as the Nature Oasis (Lake) of Angitola (875 ha), although placed outside the territorial continuity of the Park area. Belonging to the Serre Park are 26 municipalities, whose territories fall in the provinces of Catanzaro, Vibo Valentia and Reggio Calabria, in the former mountain communities Serre Calabre, Alto

Mesima, of the Ionian Slope, Stilaro-Allaro and in the agrarian regions Montagna di Serra San Bruno, Colline Occidentali del Mesima and Colline Orientali del Mesima e dell'Angitola; Agrarian region; Montagna di Soverato and Colline Litoranee di Soverato. From the identification of land values are derived the guidelines for protection and control, prohibited and permitted uses through zoning:

- Zone A (integral reserve), with an area of 236 ha, encloses the highest naturalistic values: part of the SCI Bosco Archiforo, the Bosco di Santa Maria and Monte Pecoraro.

- Zone B (general oriented reserve), a protection regime that includes regional forests and perimeter strips contiguous to Zone A. No land transformation is allowed in it, but traditional agro-sylvo-pastoral activities can be practiced.

- Zone C (protection area), with an area of 12,142 ha, includes municipal and/or other state-owned properties and private agricultural properties. Agricultural activities according to traditional uses or with organic system, silvo-pastoral activities, handicrafts and collection of natural products can be practiced in it.

- Zone D (development area), where the greatest human presence is, covers an area of 50 ha. It covers urban, peri-urban, expansion and adjacent areas, and areas designated for touristic and production settlements. Activities aimed at the sociocultural improvement of local communities are allowed in this area, subject to the indications contained in urban planning instruments.

- Oasis (Lake) Angitola, includes the lake of the same name and strips with terms of respect.

1.2.1. Geomorphology and climate

The Serre Park is distinguished as a result of geological peculiarities that allow the natural environment to be enhanced; there are arid clayey gullies, isolated granite masses emerging from the dense forest, lacustrine depressions, and the courses of the eastern torrents. The morphology presents a generally gentle and regular course, and it is only adjacent to the numerous watercourses that distinguish all areas that even very steep slopes are observed. The watercourses, which branch off from the long relief of the Serre, take on two different connotations: the Ancinale represents the axis of longitudinal structure of the double system of the Serre; while the Assi, Stilaro and Allaro descend more steeply toward the undulations of the Ionian coast, where the plateau presents a strong escarpment. The Mésima, with its 800 km^2 basin, is the most important river on the western side of Calabria, and the Marepotamo River flows into it.

Stilaro River, which gives rise to the spectacular Marmarico Falls, which, with a drop of about 100 m, are the highest and most impressive in all of Calabria assuming considerable importance for its naturalistic and environmental features. Although they are not high altitudes, the agre mountains present host lush forests of beech, oak, silver fir and chestnut trees.The landscape is characterized by areas of particular naturalistic interest: the Archiforo Forest, dominated by majestic white firs and imposing beech trees and intersected by a network of nature trails; the Biogenetic Reserves of Marchesale and Cropani Micone, which together with the other Calabrian forests constitute a heritage of considerable biogeographic value; the Lacina Plain; and the Angitola Oasis. The anthropized landscape is fragmented into small settlement contexts, because the organization of living spaces has been affected above all by the altimetric characteristics, morphological connotations and physical configuration of the territory, which has determined a comb-shaped settlement pattern shaped by the hydrographic system that develops in parallel and close paths. Even the centers lying in the coastal plains, despite being more easily contaminated by the well-known phenomenologies of urban transformations, have retained some landmarks. The whole takes on in the historical-environmental landscape unity a "choral value" that shapes a harmonious reality. The climate on the Serre Calabre plateau is Mediterranean in nature with mild winters and hot summers; it varies with altitude and from the slopes. According to Rivaz-Martinez's (Quézel et al., 1995) classification, the macroclimate falls within the oceanic temperate type, the thermotype is mesotemperate, and the ombrotype is hyperhumid (Mercurio & Spampinato, 2006). Air humidity is consistently high throughout the year, and even on the hottest days the maximum values are very close to 100 percent so that, frequently, there is the formation of fog and occult rain. Only in the hottest hours of the day is the humidity rather low (Iovino et al., 1988, 1989). The abundant rainfall throughout the year, together with favorable air humidity conditions, means that soil moisture conditions are always above the wilting point even in the absence of rainfall for rather long periods, so that forest vegetation is hardly subject to stress phenomena (Iovino et al., 1988; Iovino et al., 1989). De Martonne's aridity index, always above 60, confirms particularly favorable conditions for forest vegetation. According to Pavari's phytoclimatic classification (Pavari, 1916), the areas considered fall, at lower elevations, into the cold subzone of the Castanetum. The higher elevations, on the other hand, can be ascribed to the warm subzone of Fagetum. From the phytosociological point of view, the stands can be framed within the Anemono apenninae-Fagetum (Gentile, 1970), the Aquifolio-Fagetum (Gentile, 1970; Mercurio & Spampinato, 2006) and the Anemono apenninae-Fagetum abietosum albae (Caminiti et al., 2002) when in addition to beech, fir and sycamore (*Acer pseudoplatanus* L.) are present. Among nemoral species are frequent *Ilex aqui- folium* L., *Melica uniflora* L., *Daphne laureola* L., *Lathyrus vernus* L., *Anemone appennina* L., *Cordalis solida* L., *Scilla bifolia* L., *Allium pen- dulinum* Ten., while *Geranium versicolor* L., *Lamium flexuosum ssp. pubescens* Ten., *Doroni- cum orientale* Hoffm., *Festuca exaltata* C. Presl., Parl., *Luzula sicula* Parl., *Digitalis micrantha* Roth., *Galium odoratum* L., *Viola reichenba- chiana* Boreau, *Sanicula europaea* L., are indicative of some environmental thermophilicity. The Tyrrhenian foothills, those near the Mesima and Angitola valleys, have a milder Mediterranean climate than the Ionian side, where there is less rainfall and higher temperatures. In the central valley areas, which correspond to the basin of Serre San Bruno, Mongiana and Fabrizia (between 800 and 1,000 meters), the climate is a transitional Apennine Mountain type (a middle ground from upper mountain to chestnut foothills): thus, there are cold and rainy winters, but also with snow during the coldest periods with an average of at least two snow events per year, and hot, but not droughty summers. Finally, the mountain area from 1,000 meters and above (the summit is 1,423 Mt. Pecoraro) has a typical mountain climate, characterized by fairly cold, foggy, and snowy winters with snow accumulations that persist into spring, cool and relatively rainy springs and autumns, and sunny but cool summers with some morning fog and a few afternoon thunderstorms. The climate in the northernmost belt of the Serre (ridge of Mount Cucco, Mount Pizzinni, Serralta di San Vito) has a particular more humid microclimate, thanks to the greater proximity of the Tyrrhenian and Ionian Seas, favors mountain vegetation at lower altitudes than usual. Chestnut thus immediately gives way to beech as early as 700 meters. (See Monte Cucco beech forest). The rainfall in the area is above the regional average. In the western greenhouses, rainfall reaches 1,100 and 1,400 mm annually, while in the eastern part it is significantly lower.

1.2.2. Forest types of the "Serre" Regional Park

The Park area is characterized by the widespread presence of forests and woodlands, Mediterranean scrub, pastures, and agricultural crops.

The predominant tree species are: *Castanea sativa* Mill. (European chestnut), governed by tall trees in the best areas and coppice in the others, *Pinus nigra* Arn. subsp. laricio Palib. ex Maire (Laricio pine), *Alnus glutinosa* (L.) Gaertn. (Common alder), *Alnus cordata* (Loisel.) Duby (Neapolitan alder) in spontaneous thickets covering all valley parts, cooler areas and riverbeds, *Fagus sylvatica* L. (Beech) to *Abies alba* Mill. (Silver fir) present in groups and more or less extensive pure and mixed forests in the higher and cooler areas, *Quercus ilex* L. (Holm oak), *Populus alba* L. (White poplar), *Populus tremula* L. (European aspen) and *Robinia pseudoacacia* L. (Black locust) at a sporadic level in the lower areas. The shrub vegetation is rich in the most typical essences of the Mediterranean maquis: *Cytisus scoparius* L. & Link, *Calluna vulgaris* L., *Arbutus unedo* L., *Myrtus communis* L., *Phillyrea angustifolia* L., *Cistus salviifolius* L., *Pistacia lentiscus* L., *Ilex aquifolium* L., etc. The natural stand of the Park territory is also interesting for the ecological and silvicultural characteristics of Silver fir.

1.3. Silver fir (*Abies alba* Mill.)

Silver fir, as a result of its distribution, landscape contribution, naturalistic and productive function, as well as its balancing role played within forests, is classified as one of the most important species found in Europe. In the Continent of Europe, there are more than 50 different species belonging to the genus *Abies alba* Mill., in particular, *Abies sibirica* Ledeb and *Abies balsamea* Mill. stand out due to their greater presence (Figure 3).

Figure 3: *Silver fir stand (Abies alba Mill.).*

Within the National Forest Inventory are reported 29,700 ha of forest stands with a predominance of silver fir (*Abies alba* Mill.), which in Italy is found mainly in Trentino, Tuscany, Marche, Molise, Basilicata and Calabria. Silver fir is a conifer belonging to the Pinaceae family; it possesses very regular branching, with whorl-like branches extending horizontally. The main evergreen branches are grouped in regular antlers arranged along the stem, which is cylindrical, long and straight. In uneven-aged forests, the palques branch off from about half the height of the stem. In contrast, secondary twigs are arranged along the stem in a spiral pattern. During the juvenile stage, the fir tree has a rather conical crown, smooth bark with light coloration and with resin blisters, while with increasing age the crown takes on a more flattened shape, resembling a stork's nest, and the bark becomes fissured, taking on a more brownish coloration. Despite the resin pockets on the young bark, there is a total absence of resin on wood, that makes this species particularly useful for interior environments like floors, coatings, doors, windows. In addition, the color remain clear longer than pine or spruce wood.

Silver fir is an early tree reaching 55 (60) m in height, and 2-3 m in diameter (Farjon, 2017). The persistent leaves are solitary and arranged in a comb-like arrangement along the twigs, with two pale somatic lines on the lower page. The flowers, united in unisexual strobili, are distinguished into yellowish-green male flowers and female flowers with reddish-purple coloration. The cones are always erect, oblong-cylindrical in shape, with deciduous covering scales. The relatively unbranched root system is taproot type and reaches a depth of 1.60 meters. Among conifers, it is one of the species that best anchors itself to the soil and is therefore less susceptible to breakage or uprooting caused by storms. The fir tree is a tall resinous tree whose trunk rises far from the ground and tends to the sky, thus, to God, in the quest for light. Snow-white and silent, winter, like a mantle, caresses the shady mountain woods. Conifers, moved by the frosty air, sway at the apex, and soft snowflakes descend gently to enchant the earth. In the heart of winter, while so many trees shed their leaves, Nature sleeps and life slumbers, the Fir trees, on the other hand, "keep watch" The Spruce retains its deep green, needles and thick, hardy foliage. This characteristic of it, similar to that of other evergreen trees, was interpreted by the ancients as a symbol "of immortality, of pulsating life" which endures unchanged beyond the cycles of existence on earth; beyond the sleep and awakening that follow one another incessantly. The fir tree is symbolically linked to the winter solstice because it recalls the "deep regeneration, the blossoming of luminous life in the center of darkness" and, therefore, the birth of the Divine Child and the shining Sun whose path of descent into the depths of the earth ends on the longest night of the year and that of emergence begins, concomitantly, with the lengthening of the length of the day. It is a mesophilic, late-successional species, becoming dominant or co-dominant where mean annual rainfall exceeds 600-700 mm and summer rainfall 120-150 mm. The 600 mm threshold marks the lower limit in the natural range of vegetation. Silver fir is a species that tolerates shady environments very well, related to the climatic horizon of broadleaf sciaphilous trees (lower horizon of the mountain plateau). Silver fir was naturally mixed with beech in the ancient mixed forests that are unfortunately disappearing today.

1.3.1. Range and autoecology of silver fir

The range of silver fir is mainly mountainous except for two relict areas, one in Normandy and one in Poland near Warsaw, respectively. Remaining included are: the Pyrenees, the French Massif Central, the entire Alpine system, the Vosges, the Dinaric Alps, and the entire Carpathian system as far as Romania. To the south:

Corsica and the Apennines to Aspromonte. The places of greatest concentration are the Vosges and Jura, the middle mountains of Switzerland, the Black Forest in Germany and the Eastern Alps also in Italian territory.

In the postglacial, silver fir spread northward from multiple refugia. The stream of Apennine origin was most effective in restocking throughout the Alps and middle Europe (Kral, 1989). For a fairly long period, silver fir had a considerable frequency and even a strong push to subalpine altitudes, thereafter, the period of retreat and decline in abundance began, in favor of spruce or beech according to elevations and locations. Since this decline began very recently (2000 to 600 years ago according to locations) it remains uncertain whether the causes are natural or anthropogenic. In Italy, silver fir covers an area of more than 68,000 ha, of which about 42,000 consists of coniferous forests; this species, is particularly frequent in the outer edge of Trentino, the Asiago plateau, Cadore and the Carnic Alps. In this area there are many mixed forests with spruce and beech treated with occasional cutting and of considerable productivity (Del Favero, 1990). In the remaining part of the Alps, silver fir is still found mixed with spruce and beech (in Piemonte Regionwith larch and beech), taking into account that, the appearance of the species is more fragmentary. In the Apennines, it is possible to denote a greater dispersiveness of current silver fir stands; In fact, more than fir stands, historical records of the species' retreat resulting from pollen analyses (Ferrarini, 1962), or from archival documents or data from toponymic records (Gabbrielli et al., 1990; Gradi, 1983) abound. In Tuscany, silver fir was grown on grand ducal or monastic estates and was considered the symbol of "spiritual elevation and meditation," so it was cultivated in all the gardens of monks' convents since long time. The link between the fir tree and the monks was so strong that an ancient saying went, "*ubi fratres, ibi abies*," "where there are monks, there is fir" (Marziliano et al., 2011); thus, the species has been preserved, but separately from its original consortia. A core of spontaneous fir can be observed south of Mount Amiata in a typical downslope position mixed with cedar oaks (Negri, 1943). In the Marche region a silver fir station is reported in the Laga Mountains (Orsomando, 1974). The wild populations of the central and southern Apennines can be grouped into the following divisions (Borghetti & Giannini, 1984): 1) the Teramo and Gran sasso Apennines; 2) Molise

Apennines, in the Sangro Valley and in the Trigno Valley where descending positions in turkey oak forests prevail; 3) Mount Pollino; and 4) Calabria: Sila piccola, Serre and Aspromonte. Calabrian groupings are given special importance for the extent of the stands, many of which are in consociation with beech, and for the vitality of local provenance.

Silver fir, located at elevations around 2,000 m, has a high resistance to winter lows, down to - 25° C, however, its expansion is prevented by increased summer heat requirements and greater sensitivity to physiological aridity and frozen soil (Lingg, 1986). It is generally found in areas where average temperatures remain in a range of 6° C to 11° C, similar to those of beech; therefore, it is a species that is little susceptible to spring frost, but limited greatly by summer temperatures, and it fears excessive atmospheric moisture, which, among other things, favors pests (Susmel, 1959). The combination of the need for summer heat and the aversion to high atmospheric humidity cause a contradiction to the distribution of silver fir as a result of the mass altitudes of the mountains in which it is found.

Silver fir has transpiration parameters similar to those of spruce and significantly lower than those of beech. As a result of the depth of its root system, although it makes it a species adapted to withstand aridity, drought crises are aggravated by the intervention of pests. The species discussed, grows best in areas where the range of annual rainfall is 1,500-2,000 mm (in Calabria for example, this value is about 1,800 mm), is also widespread in areas where there is only 600 mm of annual rainfall, but with lower than the average temperatures, which mitigate the actions of pests.

The consequence of annual drought is found in a marked reduction in growth (Spiecker, 1986); while, according to (Calistri, 1962) rainfall in May and June are visually decisive. In addition, sequences of drought years appear to be correlated with particular disease crises (Biraghi, 1949; Moriondo & Covassi, 1981; Sibilia, 1952).

A first category of parasites includes insects (*Scolytidae* spp., bark beetle) or fungi that carry out or begin their cycle under the bark. Corticolous pests are more likely to be "weakness pests" because their action is facilitated when dryness causes loss of adhesion between the bark and the wood. The basidiomycete *Heterobasidion annosum* (Fr.) Bref. (*Fomes annosus* Cooke) is the agent of root rot in many species (Capretti, 1986). On fir it is particularly active in warmer stations. Root and butt rot causes a reduction in diametric increment and facilitates wind uprooting (Cantiani, 1967). This pest, in addition to weakening, aggravates the effects of aridity through reduction of the root system; it attacks smaller diameter plants first, and it would appear that regularly thinned fir trees are less susceptible (La Marca, 1979).

1.4. General objectives of the research activities

In order to assess whether areas mixed with species dominated by silver fir and beech are more protected from *Heterobasidion* spp. root transmission than monospecific silver fir dominated forests, probably due to the lower frequency of root anastomoses, six experimental sites were established in the area of the “Serre” Regional Park (Calabria Region, Southern Italy). The study areas belong also in two Special Areas of Conservation of the Natura 2000 Network. Two forest types were identified: a mixed stand of silver fir and beech located in the 'Archiforo' forest and a pure silver fir forest stand in the area of 'Santa Maria', stand. Both the studied stands are located in the municipality of Serra San Bruno (VV). The two forest types were chosen trying to minimize the differences among the environmental factors, with the aim to make the mixing effect the main ecological factor discriminating the two studied conditions. In fact, the sites have similar site conditions in terms of geographical location, topography, aspect, climate, bedrock, and soil type. In order to quantify the mixing effects, the pure stands were used as reference for the mixed-species stand. The pure stands were selected when the corresponding species represented ~90% of the stand basal area. The mixed forest was defined as the stand in which the two species of interest together represented at least ~90% of the total stand basal area. Before starting with the specific field study, a deep literature research activity was realized, with the aim of writing a review of the state of the art concerning the relationship between the *Heterobasidion* spp. occurrence and the forest growth dynamics, with particular focus on mixed and pure forest stands.

In addition, attention was focused to the growth patterns of silver fir as a function of the presence-absence of mixing with beech. In this context, within the “Serre” Regional Park, through dendrometric analyses and dendrochronological approaches, it was tested whether the mixing of spruce and beech species can improve the growth pattern and fitness of silver fir compared to the pure stand, assuming that the presence and propagation of roots infected by *Heterobasidion* spp. may be limited by species mixing, thus reducing its propagation through trees; this is because the fungus also tends to spread through anastomoses, i.e. through root contacts (Asiegbu, 2000; Brūna et al., 2021).

The third objective of this study was to verify if the quality of woody products and their biomechanical characteristics can be improved by the promotion of mixing between tree species, with particular reference to pure and mixed forests of beech (*Fagus sylvatica* L.) (FS) and silver fir (*Abies alba* Mill.). Particularly, considering that the silver fir growing in mixed stand was less affected by *Heterobasidion* spp. root occurrence and propagation, we hypothesized a better quality of the silver fir woody materials when deriving from mixed stand with beech. In order to verify this hypothesis, Non-destructive methods (TreeSonic timer) useful for estimating the Dynamic Modulus of Elasticity (MOEd) and infrared spectroscopy (TF-IR spectrometer) useful for estimating the presence and abundance of molecular components such as lignin and cellulose. For these purposes, also a LDA+clustering model was applied. In addition, an attempt will be made to construct a model based on linear discriminant analysis and developed in Python to distinguish different forest types on the basis of the IR spectrum.

CHAPTER 2

2. *Heterobasidion* spp. and forest health: a review

Abstract

Heterobasidion spp. is a species complex comprising five species that are widely distributed in coniferous forests of the Northern Hemisphere, each characterized by a distinct host preference. Many papers have been published on these fungi in the past four decades, making them perhaps the most widely studied forest fungi. *Heterobasidion* species are at different levels on the saprotroph-necrotroph gradient, and the same individual can switch from one mode to the other. The abilities of *Heterobasidion* spp. to infect woody materials and to spread to neighboring trees as pathogens have resulted in significant damages to timber production in managed forests. This review, based on classical and modern studies, focuses on the current knowledge of the taxonomy and distribution of *Heterobasidion* spp., its genetics and life cycle, epidemiology, dealing then the economic and ecological impact on forest productivity.

Keywords: *Heterobasidion* spp., Woody materials, Forest productivity, Ecological impacts, Forest health, Managed forests.

2.1. *Heterobasidion* spp.

In European forests and, more generally, in the coniferous forests of the northern regions of the world, losses of both economic and ecological value are increasing due to higher frequency of extreme events such drought, storms, pathogen attacks and forest fires. The species, the properties of a stand, the health condition of the trees, as well as the anchorage of the root system and the characteristics of the aerial part of the plant together determine the tree stability. In fact, the presence of disturbance agents, which could be pathogens such as *Heterobasidion* spp., tends to amplify the susceptibility of forests to damage caused by extraordinary events, effectively increasing the vulnerability of forest stands (Asiegbu et al., 2005; Brūna et al., 2021; Krisans et al., 2020). Important fungal pathogens and in particular species of *Heterobasidion* spp., a basidiomycete fungus belonging to the family Bondarzewiaceae and typical of northern temperate and boreal forests, cause rot to the hypogean and epigean parts of more than 200 different species, including many broadleaf and some non-woody plants but, they attack conifers the most, proving damaging to merchantable wood and responsible for severe annual economic losses ranging from 50 to 800 million euros in Finland and Europe alone (Vainio & Hantula, 2016; L. Wang et al., 2014) but no less important are the losses at the ecological level given the fundamental role forests play in carbon dioxide capture and sequestration (CCS) (Oskars, 2020), as well as the losses of the new recreational and landscape functions assumed by mountains (Figure 4).

Figure 4: *Heterobasidion spp. presence at the trunk base of a coniferous tree.*

2.2. Taxonomy and distribution

Paleobotanical studies of fungal fossils dating back to the Triassic (200-250 million years ago) have shown that there was already the existence of a fungus very similar to *Heterobasidion annosum sensu lato (s.l.)* at that time and it was widely spread in epidemic form in American lowland forests (Creber & Ash, 1990). In Trentino, from an ancient document dated 1568 by the Sienese botanist Pietro Andrea Matthioli, many similarities were found between the symptomatology of *H. annosum s.l.* and the disease detected by Matthioli himself. The Sienese botanist in repeated trips made to the forests of the valleys of Non, Fiemme and Sole, on behalf of Prince-Bishop Bernardo Clesio, although without recognizing the infectious agent, described the symptoms on the pine of a serious disease well known to the local mountain people. After this report, which likely referred precisely to *H. annosum s.l.*, we have to wait until 1800 for a complete description from another Italian, Abbot Fornaini, who mentions this pathogen as widely present in the artificial silver fir plantations of Vallombrosa (Fornaini, 1804, 1825).

Heterobasidion annosum (Fr.) Bref. is a phytopathogenic fungus first described and classified by Elias Fries in 1821 under the binomial *Polyporus annosus* Fries. It was later reclassified under the names *Trametes radiciperda* Hartig in 1878, *Fomes annosus* Cooke in 1885, *Heterobasidion annosum* Brefeld (1889), *Ungulina annosa and Fomitopsis annosa* in 1941. The conidial form was first named Oedocephalum lineatum by Bakshi in 1950, then later Spiniger meineckellus (Olson) by Stalpers in 1974 (Hanlin, 1982).

For a long time, the fungus was observed and studied as a single species until mating experiments revealed intersterile groups (IGs), which differed according to the host in which they settled, within *Heterobasidion annosum s.l.* It had to wait for the contribution of (Korhonen, 1978), who unequivocally established the existence of 2 host-specific races of *Heterobasidion annosum s.l.* He studied the phenomenon of mating and karyogamy through sexual compatibility experiments between isolates from different hosts. He used 780 Finnish isolates plus 80 others from various parts of the world and from various hosts. In this study he pointed out an obvious manifest sterility at mating of isolates that showed a marked preference for the host species *Pinus* spp., which he called "P" with those isolated from *Picea abies* (L.) Karst, and which preferred this host he called "S" from the English "spruce." From his results the S group was strictly limited to *Picea abies* with very few exceptions. It was rarely found in pine seedlings adjacent to infected *Picea* stumps and in any case never in adult pines. Group P was commonly found in pines of all ages, but it was also isolated a few times on Juniperus communis, *Fagus sylvatica* L., *Betula* spp., *Alnus* spp., *Calluna* spp. and sometimes on *Picea abies*. Comparing the 80 isolates from various parts of the world he found the S group to be found in Norway, Sweden, Denmark, Germany, Italy, India and Japan. Group P was found in Norway, Denmark, Scotland, France, Italy, Portugal, Canada and the

United States. Other cultures received from Australia, New Zealand, Fiji, and Japan were not compatible with either group. In the same study, (Korhonen, 1978) showed that the two intersterile groups P and S could also be statistically distinguished by other morpho-physiological features besides host-specificity. In fact, these two groups had distinct characters such as porosity of basidiocarps, staining of mycelium grown on agar-malt substrate, growth of mycelium at different temperatures, size of conidia, competition to *Phlebiopsis gigantea* (Fr.) Jül.

In Italy, in addition to the presence of the previous 2 groups, the presence of a third host-specific intersterile group of *Abies alba* Mill. had already been hypothesized by Moriondo et al. (1988). Korhonen et al. (1989) with the analysis of a sample of 65 Italian isolates, corroborated the hypothesis of the existence of a third intersterile group which they called "F" from the English "Fir". This Italian specificity had also been inferred on the basis of a relative resistance of *Abies alba* Mill. toward attacks of the pest observed in countries north of the Alps (Delatour, 1972; Yde-Andersen, 1968), and instead, of its high susceptibility throughout the Italian peninsula (Biraghi, 1949). (Capretti, 1990) confirmed the existence of group F on *Abies alba* Mill. in Italy based on a sample of 260 isolates collected from the Alps to Calabria. They also verified a curious phenomenon: the high compatibility of the F-group isolated in Italy with the Finnish S-group. Compatibility that, on the other hand, was very low between the same F and the Italian S. Based on the above, the intersterility groups were classified as types P, S and F. In Europe, type P attacks many conifers and deciduous trees but prefers Scots pine (*Pinus sylvestris* L.). Type S shows a specialization for spruce (*Picea abies* L.) by being found more in areas where spruce grows naturally. The main host of type F is silver fir (*Abies alba* Mill.), although it can also infect other hosts it is localized more where *Abies* species grow naturally. In addition to Europe intersterile groups have been identified in North America, specifically the P group that attacks mainly pine (*Pinus*) and the S/F group that prefers fir species (*Picea, Tsuga,* and *Abies*) (Asiegbu et al., 2005).

Fungi belonging to the species *Heterobasidion* spp., are pathogens that can be particularly damaging, especially in managed conifer stands, and although most likely the center of evolution is in East Asia, its speciation has occurred in Europe (Brūna et al., 2021; Vainio & Hantula, 2016) where three native taxa of the forestdestroying pathogen *Heterobasidion annosum sensu lato (sl.)* have been found : *H. annosum sensu stricto (ss.)* from all over Europe, *H. abietinum* Niemelä & Korhonen originating from southern and central Europe, and *H. parviporum* Niemelä & Korhonen originating from Europe and Asia (Dalman et al., 2010; Ioos et al., 2019); a fourth invasive allochthonous taxa was first reported in Italy in 2002 in the Presidential Estate of Castelporziano, near Rome, under the name *H. irregolare* (Gonthier et al., 2015; Ioos et al., 2019) native to North America where it had been detected and studied together with a fifth taxa named *H. occidentale* native to North America, Canada, and Mexico (Dalman et al., 2010), to date the area of infestation covers an area approximately 103 km long (Gonthier et al., 2014). On the basis of its current impact in Italy and also on the basis of its potential continental-scale effects in pine stands, *H. irregularis* has been placed on the A2 list of organisms recommended for regulation by the European and Mediterranean Plant Protection Organization (EPPO) (Garbelotto et al., 2022). Most likely the introduction of this pathogen is due to the movement of timber by the U.S. military during World War II (Garbelotto et al., 2022). The rate of spread of the pathogen has been estimated at 1.3 km/year, and this was inferred by looking at the distance between Castelporziano and the farthest invaded site and the time elapsed since its introduction, (Garbelotto et al., 2022; Gonthier et al., 2007). The taxa listed so far are joined by two others named *H. insulare* and *H. araucaria* originating from China and Australia, respectively (Dalman et al., 2010).

2.3. Genetics and life cycle

Until 2019, to be able to specifically and individually detect *Heterobasidion* species, no test was present until in a study conducted by (Ioos et al., 2019) 10 phylogenetic markers were examined to determine specific polymorphisms for each of the four *Heterobasidion* species found in Europe. Among the 10 markers finally examined only 2 proved suitable to be able to design primers and probes for use in PCR, specifically the marker gene Mcm7 targets the DNA of *H. annosum s.s., H. irregulare and H. abietinum*, while the marker RPB1 targets the DNA of *H. parviporum*. The tests in both singleplex and multiplex reactions worked well and were validated on a variety of biological material: pure fungal cultures, wood chips from colonized trees, and fruiting bodies. The RPB1 gene had already been used as a nuclear phylogenetic marker for *Heterobasidion* spp. however, the present study confirmed the potential of the RPB1 marker gene for species identification and allowed for the successful design of specific oligonucleotides for *H. parviporum*. On the other hand, with regard to *H. annosum s.s., H. irregulare, and H. abietinum*, it was possible to design primers for real-time PCR and specific probes using the marker gene Mcm7, a single-copy nuclear gene that had not yet been used to study *Heterobasidion* spp. before this work (Ioos et al., 2019).

The species *Heterobasidion* spp. h is able form fungi characterized by a life cycle typical of basidiomycetes, this cycle is divided into two distinct and separate phases and consists of a short homokaryotic phase (where one type of haploid nucleus inhabits the mycelium) and then a heterokaryotic phase (where two or more genetically different haploid nuclei inhabit the mycelium). The formation of the fruiting bodies (basidiocarps), which are responsible for the production of sexual spores in the air (basidiospores) by the heterokaryotic mycelium takes place under appropriate environmental conditions (Van der Nest et al., 2014), the key factors, therefore, for the release, deposition and survival of spores are temperature and humidity as they are released when the temperature in the basidiocarps exceeds 0°C and deposited up to a maximum temperature of 32°C. Also crucial for spore release is the humidity of the air, in fact studies show that periods of drought slow down or even stop the production of spores that remain viable for at least 16 months (Gunulf Åberg et al., 2016). Germinated basidiospores at this point generate homokaryotic hyphae that can fuse to establish the heterokaryotic phase again. For mating and subsequent heterokaryon formation to occur, the presence of the mating-specific genes located within the mat-A and mat-B loci is required. However, the mating system essential for sexual reproduction in *Heterobasidion* spp is controlled exclusively by the mat-A locus, which is capable of encoding two classes of homeodomain transcription factors. Unlike the mat-A locus, the mat-B locus is not involved in mating recognition but is responsible for encoding G-protein-coupled pheromone receptors responsible for controlling nuclear migration and the fusion of clamp connections. A key role during mating is also played by intersterility (IS), which is responsible for preventing mating compatibility between homocarions belonging to different species, being a heterospecific type of recognition. Intersterility (IS) within the *Heterobasidion* spp. is controlled by a bi-allelic system (+ or - alleles) consisting of five genes named S, P, V1, V2 and V3. Thus, two homokaryons will be IS compatible only and exclusively if they share at least one + allele in one of the IS loci (Van der Nest et al., 2014).

2.4. Epidemiology

The fungus spreads via basidiospores, which are produced in large quantities from fruiting bodies, on strains and on wounds but also via mycelia through anastomoses, that is, provided root contacts are present (Asiegbu, 2000; Brūna et al., 2021). (Rishbeth, 1951) made one of the major contributions to the knowledge of the infective mode of *H. annosum* s.l.. According to his study, there are two modes by which the fungus spreads in a given environment (Figure 5):

1. primary-type infection that develops when basidiospores or conidia come in contact with fresh plant wounds; on the cut stumps the infection starts and spreads to the root system.

2. secondary type infection affecting standing plants, due to vegetative mycelium spreading by contact or root anastomosis from nearby stumps with primary infection.

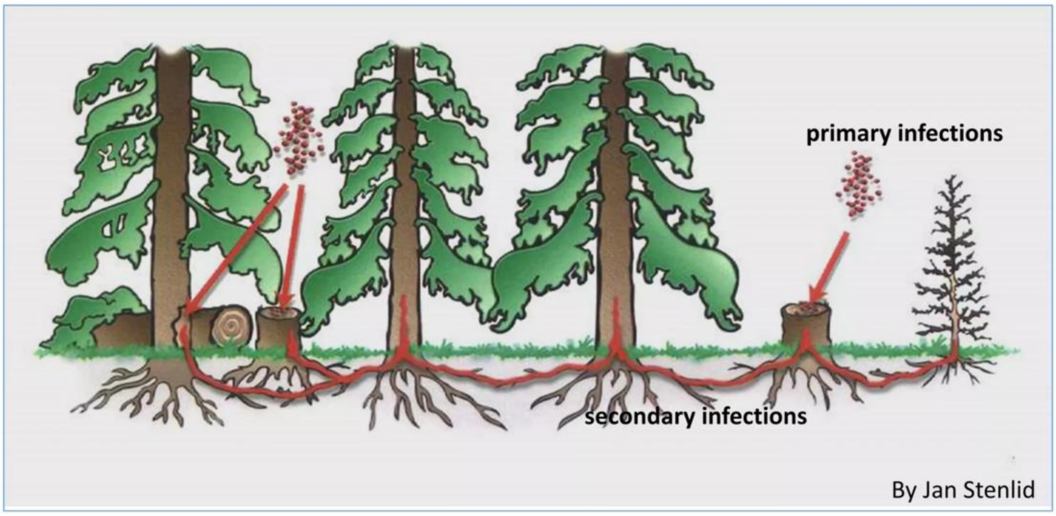


Figure 5: *Primary and secondary infections during the fungus spreads.*

The spread then occurs in a spotty manner with outbreaks progressively becoming larger (Moriondo, 1999). All hosts that are attacked exhibit root rot, but the subsequent stages of stem colonization vary according to host species. In the case of silver fir and spruce the infection, which started from the anastomoses of smaller roots with nearby infected roots, affects the wood and bark, but only up to a certain size of the root section. Beyond 3 to 5 cm, although it travels up to the collar and into the stem, it remains localized in the inner part of the wood (Shain, 1971a). In fact, the reaction of living sapwood tissues limits the action of the pathogen through the production of repellent substances such as phytoalexins, tannins, phenols, terpenoids, quinones, and resins (Shain, 1971b). When observing roots in cross section, this reaction zone is often identified by a browned band, about half a centimeter across, which is located at the outer edge of the part colonized by the fungus. The continuous action of the pathogen and its enzymatic kit, which is capable of utilizing lignin, cellulose, hemicellulose and cell wall pectins, together with the action of other saprophytes, causes complete disruption of the woody structure, which can sometimes go as far as complete emptying of the stem.

There are at least five clear steps during the decay process (Figure 6):

Figure 6: *Decay process after the fungus infection.*

The height in the stem at which decay can develop can easily reach as high as 2-3 meters in *Abies alba* Mill. (Shain, 1971a) and 10-12 in *Picea abies*. Affected plants show profound alterations in the mechanical strength of the root system, so they are more susceptible to being crashed or uprooted by wind. The latter phenomenon may occur particularly frequently in *Picea abies* attacked by *H. annosum s.l.*, given the normally shallow root system of these species (Figure 7).

Figure 7: *Aterations in the siver fir wood characteristic after the fungus spread..*

Incentivizing the spread of decaying pathogenic fungi may be especially silvicultural operations especially if conducted at temperatures above freezing point causing, therefore, airborne dispersal of spores from fruiting bodies that are able to sporulate and infect stump surfaces and lesions from forestry operations (Müller et al., 2018). Thus, for the establishment of *Heterobasidion* infection, the availability of fresh surface of woody material is of crucial importance and in this case we are going to talk about primary infection, specifically when wind-borne basidiospores of *Heterobasidion* spp. are deposited and germinate on stump surfaces or on lesions created as a result of silvicultural operations (Gaitnieks et al., 2021), at this point it can be argued that the prevalence of fruiting bodies of the fungus is correlated with the intensity of forest management as silvicultural operations cause an increase in the amount of wood residues (Gaitnieks et al., 2021; Gunulf et al., 2012). Conversely, trees in close proximity to diseased plants or stumps can be infected by mycelium through contact between roots (anastomosis) and in this case it is secondary infection (Gaitnieks et al., 2021; Gunulf et al., 2012;). To date, there are few comparative studies conducted on the frequency of fruiting bodies present on different parts of trees. Research in Germany and Finland, however, has shown that large decomposed spruce logs left in the forest as a result of forest use operations increase the risk of primary *Heterobasidion* infection due to the abundant formation of fruiting bodies (Gaitnieks et al, 2021). A study realized by Gunulf et al. (2013), on the other hand, showed that the risk of secondary infection is particularly influenced by both the diameter and age of the transferring stump and the receiving tree, this is because, according to the explanation given by the study authors, the probability of root contact, and thus disease transfer, increases as the stem diameter increases and also for a given stump size, but also the probability of infection is higher if it is a young stump than if it is an old one. Regarding the level of susceptibility to primary *Heterobasidion* infection of strains of different species Gaitnieks et al., (2021) conducted a study employing wood discs simulating conifer stumps, given the very rare possibility of the coexistence in the same forest, of Siberian Larch (*Larix sibirica* L.), *Picea abies*, *Picea sitchensis*, *Pinus contorta*, *Pinus strobus*, *Pinus sylvestris,* and *Pseudotsuga menziesii*. The objectives of the study were to determine susceptibility to primary infection by *Heterobasidion s.l.* by comparative inoculation of conidia, compare susceptibility of natural wood discs in the air in two Latvian spruce-fir forests infested with *Heterobasidion s.l.* and finally explore infection rates of wood discs at increasing distances from spore sources in these two forests to make inferences about the range of spore dispersal. Data from the work done by Gaitnieks et al. (2021) showed variations in the susceptibility of wood discs of different tree species to primary infection by *H. annosum s.l.* through comparative inoculation under controlled conditions, noting how larger areas were colonized by *H. annosum s.l.* on wood discs of *P. sylvestris* , *P. strobus* and *P. abies* and smaller on wood discs of *P. menziesii*; In addition, the results obtained by spraying wood discs with conidia suspensions again under controlled conditions but also exposing the discs to natural infection suggest a greater susceptibility to primary infection by *H. annosum s.l.* for *P. contorta* and other species of *Pinus* and *Picea*, followed by *L. sibirica*, and a lower susceptibility for *P. menziesii*; Finally, the highest infection rate of most wood discs was identified at a distance of 1 m from the infection center and consequently the infection rate significantly decreased at a di stanza of 5 and 10 m.

2.5. Economic and ecological impacts

Being a pathogenic fungus that goes to cause damage more in coniferous stands, there have been many studies over the years that have taken individual coniferous species as examples, trying to analyze what are the consequences on plants of infection by *Heterobasidion* spp, in this sense Wang et al. in 2014 dealt, through a study conducted in Sweden, with the damage that the fungus causes in Scots pine forests, arriving at the finding that the infection causes in the plants a reduced growth in terms of volume and growth and in some cases even results in the death of the infected trees. Another study, on the other hand, conducted in 2018 by Kovalchuk et al. (2018) in Finland on a stand of Norway spruce showed that the *Heterobasidion* infection develops very slowly going to cause rot within the trunk and in particular remaining confined to the heartwood, although in exceptional cases it manages to reach the sapwood, rarely causing the death of the individual (Figure 8).

Figure 8: *effects of Heterobasidion spp. on silver fir trees.*

A very interesting study based on dendrochronology and thus on the analyses of the growth rings of *Picea abies* was carried out by Gori et al. (2013) anche qui come sopra in the Italian Eastern Alps at three sites respectively divided into low, medium and high altitude. This work aimed to clarify what role climatic changes play on trees affected by *Heterobasidion s.l.* and specifically *H. parviporum*, comparing infected and uninfected trees, and to try to estimate volume losses due to the presence of the fungus in woody material. Of paramount importance was the choice of plants to sample since taking cores from dominant or co-dominant plants would have greatly reduced the possible influence of competition between individuals on ring growth. Thus, starting from the assumption that increased air temperature and the occurrence of summer dry spells at low-altitude sites tends to decrease the resistance of the eventual host and consequently trigger a process of increased mortality of plants affected by *Heterobasidion* spp., Gori et al. (2013), through the results of their work found that, indeed, at the low-altitude site there has been a high decline in growth over the past decade while at the mid- and high-altitude sites the decline has been much slower, even speculating that at the high-altitude site the fungus had been present for more than 80 years.

Climate change to date plays a key role in the relationships between pathogens and their hosts, as it is able to influence the competitiveness of a pathogen and at the same time the susceptibility of the host. Related to the above, Müller et al. (2015) conducted a study attempting to examine the respiration rate of *Heterobasidion* spp., and, in particular, *H. parviporum*, in climatically diverse environments. The study focused on three areas in Europe (southern Finland, Denmark, and northern Italy) and two locations in central Siberia (Krasnoyarsk and Irkutsk). For this work, the authors used homokaryotic strains from Norway spruce. Using linear models in the R statistical program, the differences between the respiration rates of subpopulations of *H. parviporum* were studied. The study based on the results showed that strains from different locations showed different adaptation to temperatures and consequently different respiration rates; In fact, strains from Siberian regions, at low temperatures between 2-15°C, manifested higher activity than strains from European regions. Finally, with regard to respiration rate, this increased when temperatures rose from 0 to 6°C in Siberian subpopulations in contrast to European subpopulations whose respiration rate rose when temperatures rose from 6 to 20°C. Basically, the differences found were minimal, showing instead greater differences within the same subpopulations isolated at different times, where a lower respiration rate was found at 20°C in the older isolates working on strains isolated 2-18 years ago and stored at 5°C, respectively. Thus, Müller et al. (2015) with the present work demonstrated how *H. parviporum* is able to adapt to global warming.

2.6. Monitoring and management

Currently, chemical treatment, biocontrol, and silvicultural practices represent the various methods that have been studied and adopted to try to prevent *Heterobasidion* infection especially during thinning operations in managed forests (Azeem et al., 2019). Silvicultural measures aimed at combating *Heterobasidion* spp. include strategic planning of thinning operations, post-intervention stump removal, and even a bias toward mixed rather than pure stands, the latter option also due to the fact that mixed forests turn out to be more productive and resilient than monoculture forests (Russo et al., 2019). Unfortunately, silvicultural approaches do not always meet what are to date the needs of intensive forest management (Pellicciaro et al., 2021) so one goes looking for alternative remedies or ones that can be complemented to the latter such as chemical treatment or biocontrol; About chemical treatment and biocontrol Blomquist et al. (2020) in a study published in 2020, compared the efficacy of treating strains with both urea and the fungus *Phlebiopsis gigantea*, available in different formulations including Rotstop® (Pellicciaro et al., 2021), both after precommercial thinning and after final felling on five spruce stands in Sweden. The study showed that the average efficacy in precommercial thinning was 93 percent for urea treatment, while the percentage dropped to 67.75 percent for treatment with *P. gigantea*; In contrast, no significant difference was found in final felling where both treatments showed high efficacy with percentages of 95.5-99.8 percent for urea and 90-98 percent with the use of *P.gigantea* fungus. It must be highlighted, however, that although urea is the agent for the most effective treatments against the *Heterobasidion* spp. Pathogen in precommercial thinnings, the feasibility of such practical use is influenced by other factors such as economics, practical legal and environmental footprint, the latter being a factor not to be underestimated as urea makes a significant contribution to ammonia emissions which is responsible for acidification and eutrophication phenomena and consequently involved in the formation of particulate matter (Column et al. 2008). Another very interesting and inherent study on biological control of forest pathogens and especially *Heterobasidion* spp. was published in March 2022, the work conducted by Pellicciaro et al. focuses on the biocontrol agent *Pseudomonas protegens* and the ability of the latter to produce secondary metabolites with antibiotic action such as pyrrolnitrin (PRN), 2,4-diacetylphloroglucinol (2,4-DAPG), phenazines, cyclic lipopeptides, volatile compounds and especially pyoluteorin (PLT) secondary metabolite involved in the inhibition of the pathogen *Heterobasidion* spp.. *Pseudomonas* protegens can be found commercially in the form of the bio fungicide Proradix® of which it is the active component but, studies previously done had shown how the cell-free filtrate (CFF) of the same bacterium as P. protegens managed to behave much better than the bio fungicide by getting to inhibit both 100% mycelium growth and 99% conidial germination. The study by Pellicciaro et al. (2022) found that a PLT concentration of 3.77 mg/L in the CFF of P. protegens performs significant antifungal activity in vitro against *Heterobasidion* spp. Unfortunately, given the loss of efficacy of PLT over time, in fact its efficacy begins to decline after 7 days of incubation against all *Heterobasidion* spp. species except in *H. abietinum*, they risk becoming a limitation for the use of this molecule as an antagonist of the pathogen in question. In principle, however, it appears that Proradix® could be an eventual excellent substitute for the products already in use against *Heterobasidion* spp. especially if treatments are carried out at low temperatures or if the product itself is maintained at low temperatures before use given the increased production of PLT when the bacterium was grown by the authors in co-culture with *Heterobasidion* spp. at 4°C (Pellicciaro et al., 2022).

CHAPTER 3

3. Growth trends of silver fir affected by *Heterobasidion* ssp. in pure and mixed forests in the “Serre” Regional Park (Southern Italy)

Abstract

In this study, interest was focused on the growth trends of silver fir affected by the presence of *Heterobasidion* spp..More in detail, according to the presence-absence of consociation with beech, it was hypothesized that the presence and propagation of roots infected by *Heterobasidion* spp. could be limited by the mixing of species, thus reducing its propagation through trees, then protecting silver fir trees from the infection. The study area is entirely located within the “Serre” Regional Natural Park (Calabria Region, Southern Italy). Tree volume increases and tree growth trends were investigated through dendrometric and dendrochronological analyses, considering how they are fundamental parameters for studying the productivity of forest stands. Silver fir trees revealed a higher hypso-diameter ratio in the pure forest as well as a higher density, while in the mixed forest there are fewer and larger individuals of silver fir. On the basis of the results obtained, the presence of the pathogen *Heterobasidion* spp. and, in particular, the intersterile group F (*H. abietinum*) was ascertained, and it was found that the tree growth trends were smaller in the pure stand than in the mixed stand for silver fir. In addition, the mixed stand ensured a greater increase in the volume of silver fir trees than the pure stand, thus proving that the mixed forest contributes to a more stable system, which also guarantees higher productivity.

Keywords: Tree consociation, silver fir-beech stands, forest structure, dendrochronology, fungi attacks, tree productivity.

3.1. Introduction

Mixed-species forests may deliver forest functions and services more effectively than monocultures (Gamfeldt et al., 2013; Van der Plas et al., 2016), particularly in threatened mountain environments (Cudlín et al., 2017) and in man-made mixed-species forests. Therefore, their spread is an important option to adapt European mountain forests and forestry to future disturbances and extreme events (Pretzsch et al., 2013). Mixed-species forests may show less temporal variation in growth and more stable productivity in comparison with pure stands, due to reduced tree species competition for resources (Liang et al., 2016; Yachi & Loreau, 2007). Nevertheless, contradictory mixed-species effects on tree growth have been found under conditions favoring drought stress or in dry years (Conte et al., 2018; Grossiord et al., 2014). Gebauer et al. (2012) observed that stand-level canopy transpiration was not higher in mixed broad-leaved forests than in pure European beech stands, but also that the spatial complementarity in root water uptake of mixed-species stands could be masked by edaphic conditions preventing the vertical stratification of species-specific root systems. An increase in the frequency and severity of drought events, as predicted for the coming decades (Hanewinkel et al., 2013; IPCC, 2017), will have dramatic implications for the resilience of Mediterranean mountain ecosystems, particularly in the case of forest stands with a simplified vertical structure (Altieri et al., 2018). Indeed, species-specific functional traits and allometric relations can be more important for stand water use than tree species diversity per se. Therefore, more insight on species mixing, structural diversity and forest dynamics is essential for modeling risk assessment and forest functions aimed at fostering alternative silvicultural practices in harsh environments.

Although mixed-species stands can be more productive in comparison with monocultures (Pretzsch & Rais, 2016; Zhang et al., 2012) up to 30% (Bielak et al., 2014), site conditions, stand age and tree species interactions affect these responses (Forrester, 2014; Petráš et al., 2016). Focusing on the causes of differences in tree growth and stand productivity between mixed-species and pure forests, most research addressed environmental settings and relationships with climatic conditions, species composition, mixture type, and stand age (Magin, 1954;

Pretzsch et al., 2010). Indeed, few studies exist on the quality and value of wood produced in mixed-species vs. corresponding pure stands (Saha et al., 2012). Information on how wood quality can be affected in relation to tree species composition is essential for decision making in adaptive forestry, especially where forest planning and thinning activities favour the occurrence of mixed-species stands (Petráš et al., 2016).

Liu et al. (2018) showed that spacing and thinning experiments in pure stands highlighted the strong effect of the surrounding spatial stand structure on tree growth and morphology and, ultimately, wood structure and timber quality. Chomel et al. (2014) demonstrated that, in mixed-species plantations, mixing hybrid poplar and white spruce might increase wood production of poplar in comparison with monocultures of either poplar or white spruce. Battipaglia et al. (2017) showed a considerable increase in cumulative basal area and in intrinsic water use efficiency in mixed-species stands of pedunculate oak and Italian alder, largely resulting from an increase in N fixation, which levelled off, when natural mortality or management practices decreased the competitive ability of Italian alder. In addition to stand productivity and water use efficiency, species mixtures with structural stratification may also enhance individual-tree growth rates and stem quality of species in the upper canopies, minimizing the proportion of taller species that reach the highest production (Kelty, 2006; Piotto, 2008).

In European forests and, more generally, in the coniferous forests of the northern regions of the world, losses of both economic and ecological value caused by the increasing frequency of storms are increasing over the years. The species, the properties of a stand, the health condition of the trees, as well as the anchorage of the root system and the characteristics of the aerial part of the plant together determine the stability of the individual tree; in fact, the presence of disturbance agents, which could be pathogens such as *Heterobasidion* spp., amplify the susceptibility of forests to damage caused by extraordinary events, effectively increasing the vulnerability of forest stands (Asiegbu et al., 2005; Brūna et al., 2021; Krisans et al., 2020). Important fungal pathogens, in particular species of *Heterobasidion* spp., a basidiomycete fungus belonging to the family Bondarzewiaceae and typical of northern temperate and boreal forests, cause rot to the hypogean and epigean parts of more than 200 different species, including many broadleaf and some non-woody plants; however, they tend to attack mainly the conifers, causing damages to the wood and inducing then severe annual economic losses (Vainio & Hantula 2016; Wang et al. 2014).

In this study, interest was directed at the growth patterns of silver fir as a function of the presence-absence of tree mixture with beech. In this context, within the “Serre” Regional Park, it was interesting to analyse the relationships occurring between different forest types, in particular pure stands of silver fir of natural origin and mixed stands of silver fir and beech, where the silver fir is affected by the occurrence of the *Heterobasidion* spp.. Interest was directed to the analysis of volume increments (current and percentage increment) and tree growth trends, as these have always been fundamental elements in the study of forest stand productivity and can be directly linked to the structural elements that characterize the stand. Thus, interest was focused on the growth trends of silver fir affected by the presence of *Heterobasidion* spp., according to the presence-absence of consociation with beech, hypothesizing that the presence and propagation of roots infected by *Heterobasidion* spp. could be limited by the mixing of species, then reducing its propagation through trees and protecting silver fir trees from the infection.

3.2. Materials and methods

3.2.1. Study area

The mixed beech - silver fir forest constitutes the most interesting element of the Serre Calabre. These are formations that have undergone numerous uses even in recent times but have managed to preserve their own peculiarities. Certainly, the ecological conditions of the station, judged by Chiarugi in 1936 to be very similar to those determined at the time of the maximum expansion of conifers, have a non-negligible importance on the great resilience of these coenoses. Clear evidence of this is the great ease with which regeneration is established and the vegetative vigor that is unparalleled throughout the rest of the range. These characteristics together with its resistance to abiotic damage meant that it was widely introduced in central

European nations to increase the resistance and resilience of local populations. The Serre Calabre plateau occupies the south-central portion of the Calabria region and consists of two broad chains running almost parallel longitudinally, extending for about fifty kilometers from the Marcellinara Strait to the Serra della Limina. To the east from the main chain are short transverse ridges that descend steeply to the Ionian Sea. The central part of the territory consists of a plateau located at about 800 m elevation enclosed to the northeast by the ridge of Monte Pecoraro (1423 m). Typical of the mountains of the Serre Calabre is the presence of several streams that have their mouths in the Ionian Sea.

The climate on the Serre Calabre plateau is Apennine Mountain type, with cold, wet winters with frequent snowfall from December to March and hot, decidedly humid summers. Spring and autumn are the rather cool seasons, and summers are always decidedly short. Rainfall is particularly abundant, just under 1800 mm per year, with Maximum winter is minimal and estimates quite pronounced. Precipitation above 100 mm occurs from October to April inclusive. In summer rainfall is particularly low, just 6 percent of the total year, lovino et al. (1985) pointed out that humidity is particularly high and throughout the year, even during the hottest season reaches values close to 100 percent. It thus contributes to mitigating the problems associated with the summer decrease.

The average annual temperature is just over 10 °C. Frosts are not infrequent during the winter, but the monthly average is never below zero. Frosts may occur from September until the first half of May, also causing frost damage. However, these phenomena are quite rare and do not cause particularly severe damage to the regeneration since these are complex-structured forests and regeneration is established almost exclusively within the forest.

The territory of the Serre from the geological point of view is definitely homogeneous. It is characterized by a metamorphic igneous basement belonging to the Paleozoic. The territory of the municipality of Serra San Bruno is characterized by acid biotitic, medium- to coarse-grained rocks, varying in composition between quartz-monzonite and granite. The rocks may be traversed by veins of pegmatite, less frequently of aplite; In addition, thin intrusions of altered diorite recur throughout the mass. The alteration of the rocks gives rise to the typical Sert sandstone; the alteration of feldspars not being pronounced, results instead, in the presence of unremarkable clayey substances.

The granite is sometimes minute-grained, or in large crystals; sometimes it is friable, or it is excellent for compactness and provides excellent cutting stone, widely used in the area for paving roads or for civil construction. The soils belong to the large Dystrudept group (Taxonomy, 1999). They are characterized by a dark brown surface horizon, rich in humus and organic matter. The differentiation of this horizon is due to the climatic characteristics of the area that facilitate the accumulation of organic matter. The soils are characterized by good water availability and a variable temperature throughout the year between 8 and 15 °C. At certain times of the year, mineralization activity is limited so the accumulation of organic matter undergoing decomposition in the surface horizons is favored.

3.2.2. Sampling of Heterobasidion spp. and laboratory identification

Identification of the sites and subsequent materialisation took place after a careful search for the fruiting bodies of the pathogen, *Heterobasidion* spp., which generally develop in the collar area and in particular on rotting or necrotic parts, in the inner cavities of stumps, on landed stems or on the root system of uprooted plants. Once the presence of the pathogen was established, samples of fruiting bodies were taken for laboratory analysis to identify their intersterile group (e.g. *H. annosum* s.s. or *H. abietinum*) (Figure 9). Sampling was carried out with a small knife, taking great care not to contaminate the samples. Once the samples were taken, they were dried and placed in sterile paper bags to improve their shelf life, avoid the development of mould and maintain storage.

Figure 9: *Heterobasidion spp. sampling.*

The analyses carried out in the laboratory, at the 'Edmund Mach' Foundation in San Michele all'Adige (Tn), have two main objectives: to confirm the presence of *Heterobasidion* spp. and to recognise the different intersterile groups that may be present.

3.2.3. Collection of fruit bodies

A representative fruit body of *Heterobasidion* spp. was collected in each of the six plots combinations. Wind throws, symptomatic trees and decayed stumps were checked for the presence of fruit bodies at each site visited. Care was taken to collect samples at locations at least 50 m apart to avoid repeated sampling of the same genotype and to obtain a representative collection of the local population (La Porta et al., 1998). After collection, each sample was dried in clean paper bag for approximately 72 hours at room temperature. Tiny layers of clean imenium were cut separately by scalpel from each fruit body and stored in eppendorf vials. A total of ca. 2 g per imenium was provided for each sample.

Unfortunately, it is not always easy the identification of the different species of *Heterobasidion* s.l. only by morphological traits of basidiomes (Mugnai & Capretti, 1989). To confirm the identification of the *Heterobasidion* spp. a molecular approach has been applied.

3.2.4. DNA Extraction

Samples were placed in screw cap tubes containing two glass beads and they were covered with 100–300 ll PrepMan Ultra Sample Preparation buffer (Applied Biosystems, Fostercity, CA, USA). They were run twice in the FastPrep Lysing Instrument (Q-Biogene, Irvine, CA, USA) for 20 s with the speed set to 4. After vortexing the samples for 30 s, they were heated for 10 min at 100 °C and centrifuged for 10 min at maximum speed. The supernatant was purified using JetQuick PCR Product Purification Spin Kit (Genomed, Löhne, Germany) following the manufacturer’s instructions.

3.2.5. Molecular identification

PCR-based identification methods have been described for various fungi. The most common methods are be based on Internal Transcribed Spacer (Lindqvist et al., 1998) and ribosomal RNA genes (Edel et al., 2000).

For rapid confirmation of the visual identification of *H. annosum* s.l., the Taxon-Specific Competitive-Priming (TSCP-)PCR method was used according to (Gonthier et al., 2003). For amplifying DNA, a mix of four primers (MLS, MLF, Mito 5 and Mito 7) was applied. PCR was performed in a 25-µl reaction mixture containing approximately 50 ng of template DNA, 0.5 µmol of each primer, 5× MyTaq® Reaction Buffer (comprising dNTPs and MgCl2 in a final concentration of 1 mM and 3 mM, respectively) and 1U MyTaq® DNA Polymerase (Bioline, London, UK). The PCR was amplified using a Master Cycler® Thermocycler (Eppendorf, Hamburg, Germany). The PCR programme 45a sas follows: 3 min at 95 °C, followed by 35 cycles of 40 s at 95 °C, 20 s at 64 °C and 20 s at 72 °C with a final extension of 7 min at 72 °C. Identification of *H. abietinum* was confirmed by sequencing of the ITS region (Internal Transcribed Spacer) by the DNA Sequence Service of BMR Genomics srl (Padova, Italy).

3.2.6. Forest structure and dendrochronological analyses

Two forest types were chosen (mixed silver fir-beech stand and pure silver fir stand) trying to minimize the differences among the environmental factors, with the aim to make the mixing effect the main ecological factor discriminating the two studied conditions. In fact, the sites have similar site conditions in terms of geographical location, topography, aspect, climate, bedrock, and soil type. In order to quantify the mixing effects, the pure stands were used as reference for the mixed-species stand. The pure stands were selected when the corresponding species represented ~90% of the stand basal area. The mixed forest was defined as the stand in which the two species of interest together represented at least ~90% of the total stand basal area. In order to assess forests of mixed species dominated by silver fir and beech and areas where silver fir was in a pure stand, 6 Experimental Sites located in the

“Serre” Regional Park were selected, each characterized by a triplet of plots (replications) (Russo et al. 2019) (Figure 10). Then, in total, 18 plots were materialized, extending each 4225 m², where the dendro-auxometric characteristics were sampled.

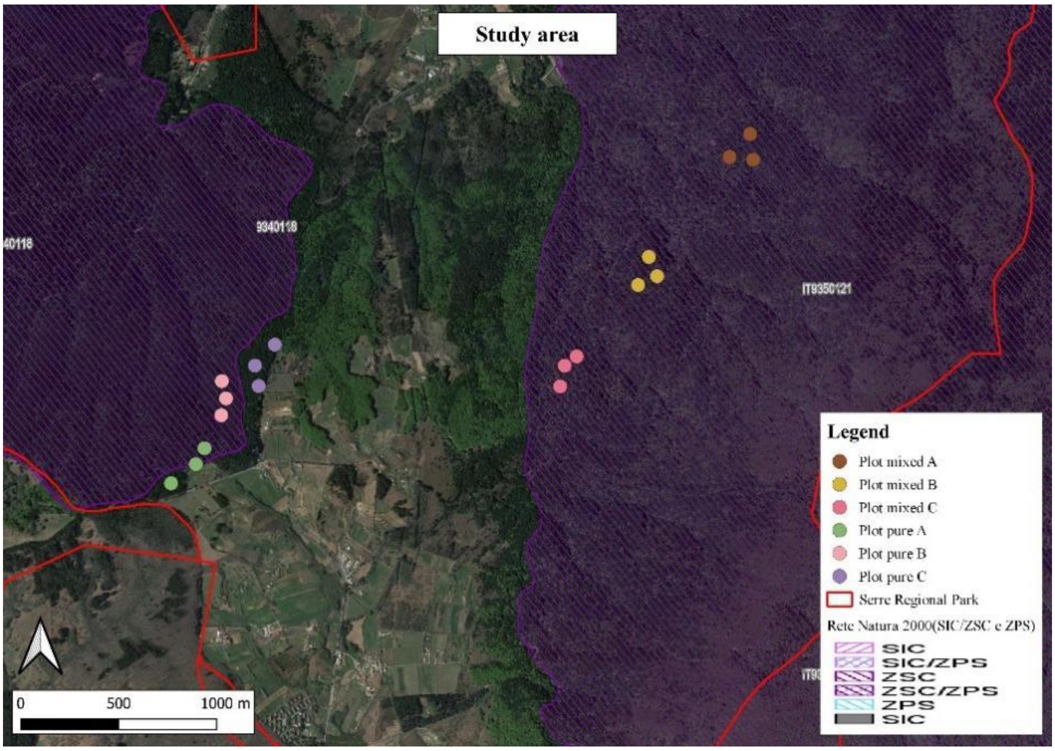


Figure 10: *The study area in the context of the “Serre” Regional Park and the plots distribution among the forest types studied.*

The plots were selected trying to detect the average conditions of the forest types studied. For the dendrometric surveys, tree diameters and position were recorded for all the occurring trees, while tree heights of at least 30 percent of the occurring trees, belonging to the different diameter classes, were recorded inside the plots. The double-entry cubature tables of silver fir and beech constructed for the National Forest Inventory (MAF, 1988) were adopted for the volume calculation.

In addition, for conventional age definition and tree growth trends, woody cores were extracted during the dormant period at 1.30 m from the ground through the use of a Pressler's increment borer; two cores per tree were extracted. (Figure 11). A total of 120 cores (20 per Experimental Site) were extracted on silver fir trees, taken 180° from each other following the direction of contour lines to ensure that compression wood was avoided.

Figure 11: *Coring of trees through the use of the Pressler's increment borer.*

3.2.7. Ring-width measurement, cross-dating procedures and statistical analyses.

The sampled cores were dried, glued onto wooden supports and finally sanded with a belt sander to make annual accretions more visible.

Tree rings were dated by counting them from bark to pith. Ring widths were measured to the nearest 0.01 mm using the LINTAB-measurement equipment, coupled to a stereomicroscope (60x magnification; Leica, Germany) (Figure 12).

The Time Series Analysis Programme (TSAP) software package (Frank Rinn, Heidelberg, Germany) was used for statistical analysis. Raw ring widths of the single series of each dated trees were plotted, checked visually and then cross-dated statistically by the per cent agreement in the signs of the first-differences of the two time series (the *Gleichlaufigkeit*, GLK) (Kaennel & Schweingruber, 1995). The GLK is a measure of the year-to-year agreement between the interval trends of two chronologies based upon the sign of agreement, or the sum of the equal slope intervals in percent. With an overlap of 50 years (which is commonly used in tree-ring studies), GLK becomes significant ($p < 0.05$) at 62% and highly significant (p

< 0.01) at 67%. With an overlap of 10 years, GLK becomes significant ($p < 0.05$) at 76% and highly significant ($p < 0.01$) at 87% (Kaennel & Schweingruber, 1995). In our work, the analyzed time series were mostly longer than 50 years and cross-dating was considered successful if GLK was higher than 60%.

The statistical significance of the GLK (GSL) was also computed. The TVBP, a Student's *t*-value modified by (Baillie & Pilcher, 1973) was used for investigating the significance of the best match identified. The TVBP is commonly used as a statistical tool for comparing and cross-dating ring widths series. It determines the degree of correlation between curves. This method eliminates low-frequency variations in the time series, as each value gets divided by the corresponding 5-year moving average.

Figure 12: *Tree rings analyses using the LINTAB-measurement equipment.*

Locally missing or discontinuous rings were identified by cross-dating the two tree-ring cores obtained from the same tree. Standard methods (Fritts, 1976) were used to build a tree averaged series and the site chronologies (Cherubini et al. 2002; Dynesius & Jonsson 1991).

The measurements were then verified using the Dendrochronology Program Library in R (dplR) package (Bunn, 2010), calculating statistics and checking the correlation of each tree with the main chronology. After validation of the data, the decreasing trend due to plant aging was removed from each ring series with an AR1 model, and the derived series was standardized with a spline curve (20-year window and 10-year overlap) to obtain the average stand chronologies, developed for each species using Tukey's robust average, which reduces the sensitivity of the calculation to outliers.

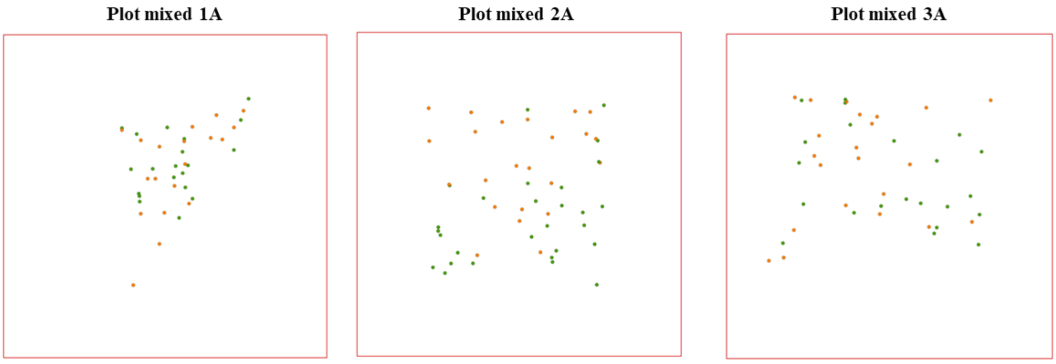
3.3. Results

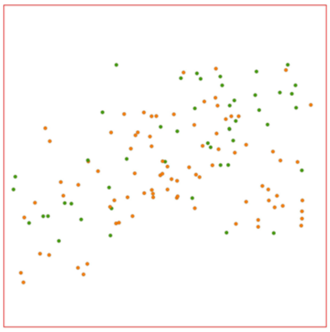
The results of the laboratory analysis are summarized in Table 1. The evaluation of intersterile groups was carried out visually on the plate on the basis of the reaction with testers. In cases where the visual method was not sufficient, the microscope was used for the detection of buckle unions. The most represented intersterile group was found to be group F (*Heterobasidion abietinum*).

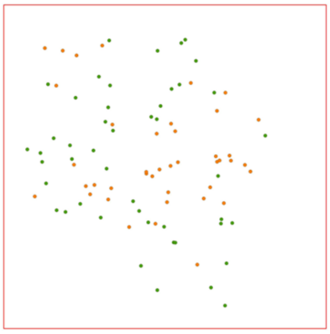
Table 1: *The results of the laboratory analysis applied for the identification of H. annosum s.l..*

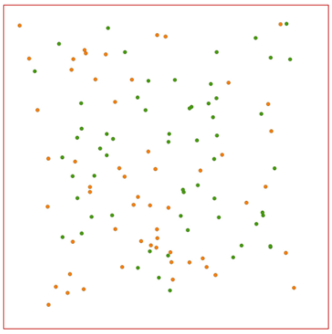
EXPERIMENTAL SITE	**INTERSTERILE GROUP**
MIXED A	F – *Heterobasidion abietinum*
MIXED B	F – *Heterobasidion abietinum*
MIXED C	P – *Heterobasidion annosum s.s.*
PURE A	F – *Heterobasidion abietinum*
PURE B	F – *Heterobasidion abietinum*
PURE C	F – *Heterobasidion abietinum*

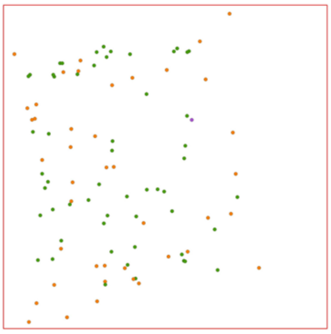
Moreover, in Figure 13, the spatial distribution of the sampled trees is reported, showing the relationships between species in the mixed stands and the tree density using a statistical approach that has already been documented in the literature (Russo et al. 2019).

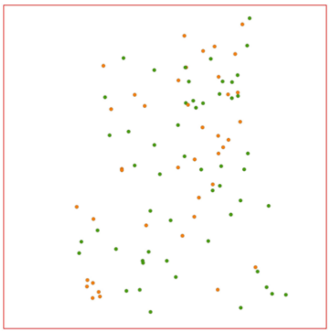


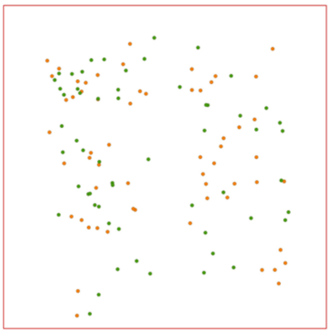

Plot mixed 1B

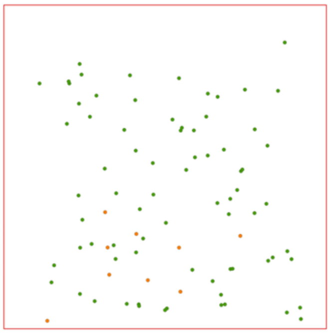

Plot mixed 2B

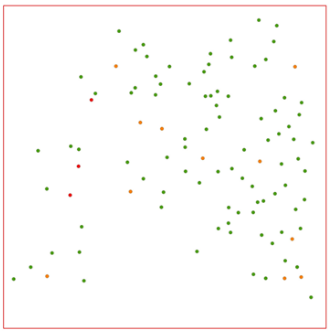

Plot mixed 3B

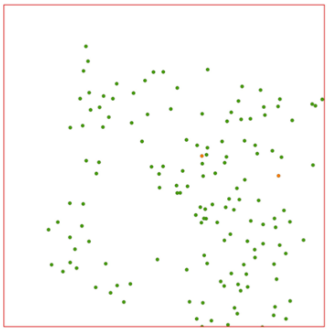

Plot mixed 1C

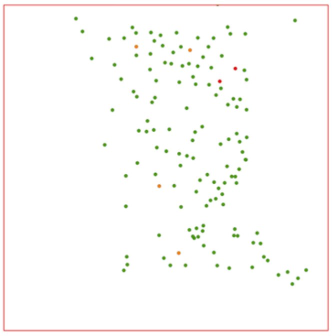

Plot mixed 2C

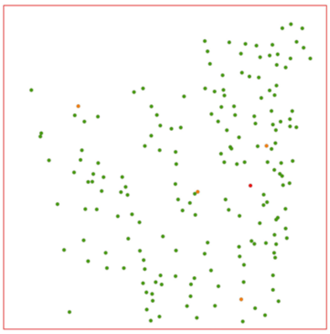

Plot mixed 3C

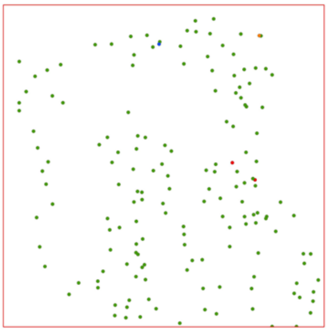

Plot pure 1A


Plot pure 2A


Plot pure 3A


Plot pure 1B


Plot pure 2B


Plot pure 3B

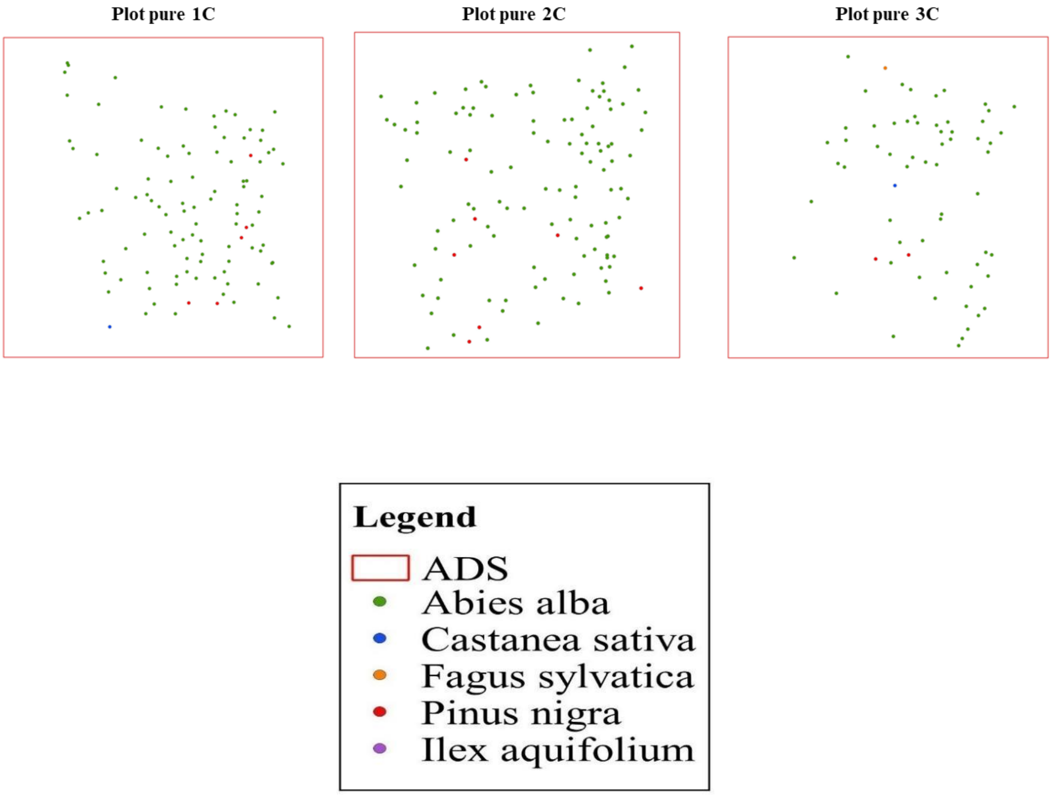


Figure 13: *Position of the tree individuals in the mixed and pure plots.*

Basing on the data collected in the field, it was possible to derive the main dendrometric characteristics for the studied stands. The hypsometric curves for silver fir, growing both in the pure and mixed stands, were derived (Figure 14 and Figure 15).

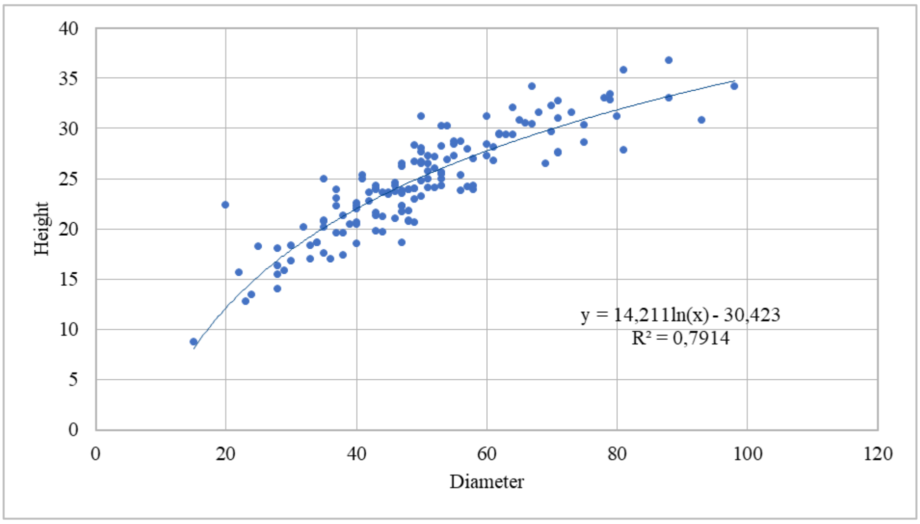


Figure 14: *Hypsometric curve for silver fir occurring in the pure plots.*

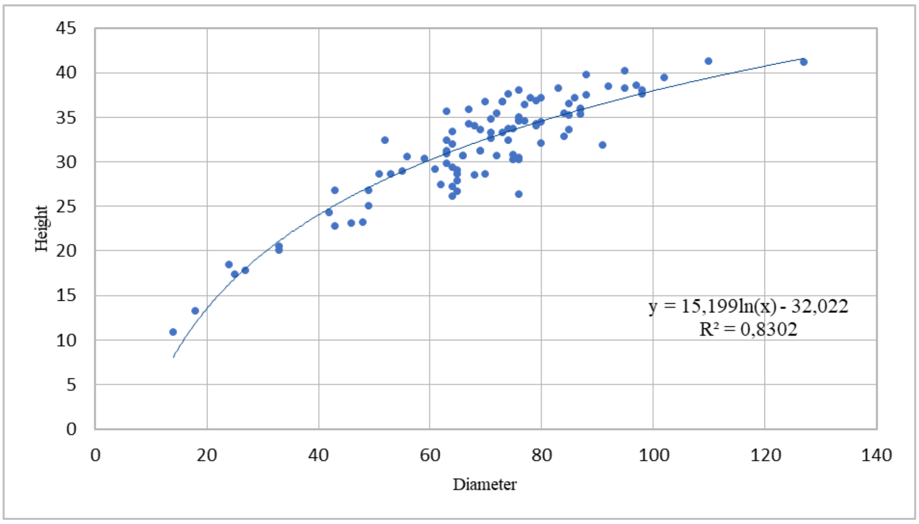


Figure 15: *Hypsometric curve for silver fir occurring in the mixed plots.*

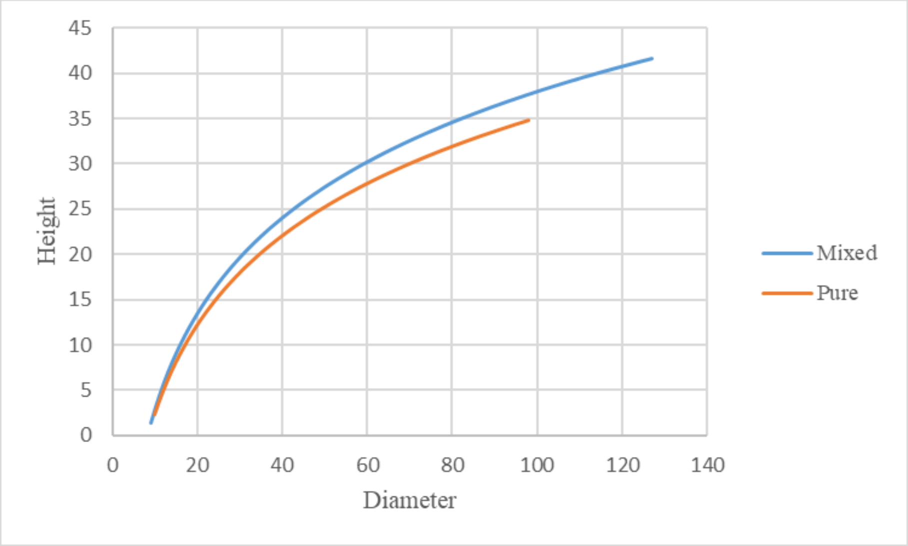


Figure 16: *Hypsometric curves for silver fir occurring in pure and mixed plots..*

The frequency distribution of plants in diameter classes was, in addition, measured. Figures 17 and 18 reveals that the diameter distribution varies from 10 to 98 cm in the pure plots while, in the mixed plots, it ranges from 5 to 127 cm, thus demonstrating that the study sites are quite fertile and that, in the mixed plot, there is a greater presence of trees with a larger diameter. A further difference, which could be observed in the two stands, relates to the phytosanitary status of the individuals: in the pure silver fir forest, most of the plants are affected by fungal infestations of the genus *Heterobasidion* spp., which impair the quality of the wood by causing rot; on the contrary, in the mixed forest, the presence of the pathogen is negligible, probably due to the interruption of root continuity between the plants.

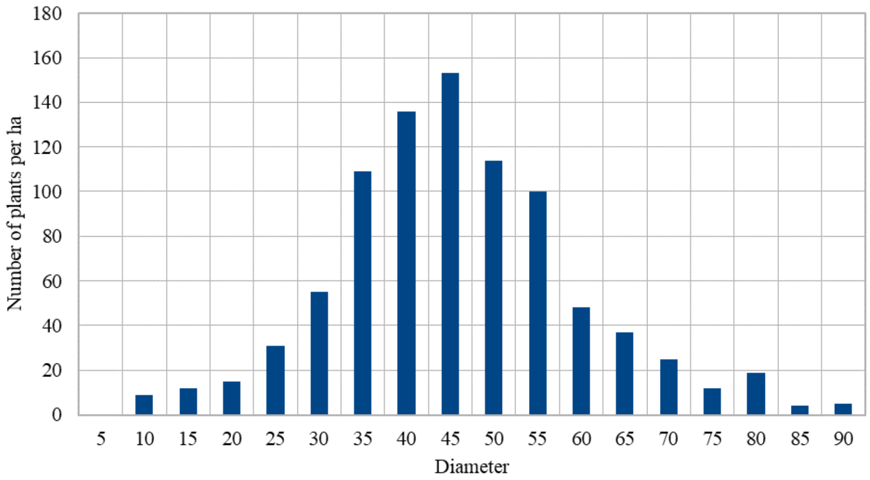


Figure 17: *Tree frequency in relation to diameter classes in the pure plots.*

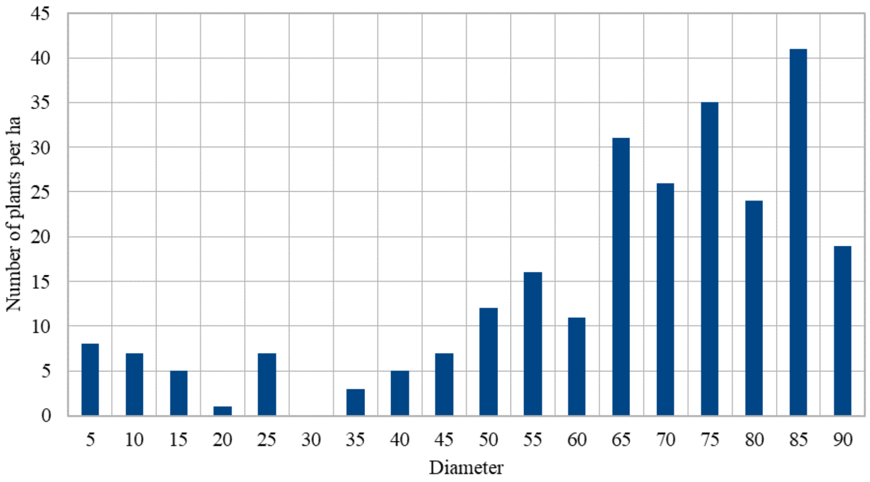


Figure 18: *Tree frequency in relation to diameter classes in the mixed plots.*

Based on the findings, the structure of the two situations (pure and mixed) was found to be significantly different by the nonparametric Kologorov-Smirnov test of equality between two distributions with the variable represented by the hypsodiametric ratio (D: 0.72 and $p < 0.01$). In fact, the plants have a higher hypsodiametric ratio in the pure forest (50.68 h/d) as well as a higher density, while in the mixed forest (47.51 h/d) there are fewer and larger individuals (Figures 19, 20 and 21). According to the measurements made, we have 307 plants per hectare in the pure stands while in the mixed stands the number of plants per hectare is 87. The estimated volume per hectare in the pure forest is equal to 58.4 m3/ha (with an average diameter of 48.3 cm and average height of 24.7 m) while in the mixed forest the volume is 187.6 m^3/ha (with an average diameter of 70.8 cm and average height of 32.7 m).

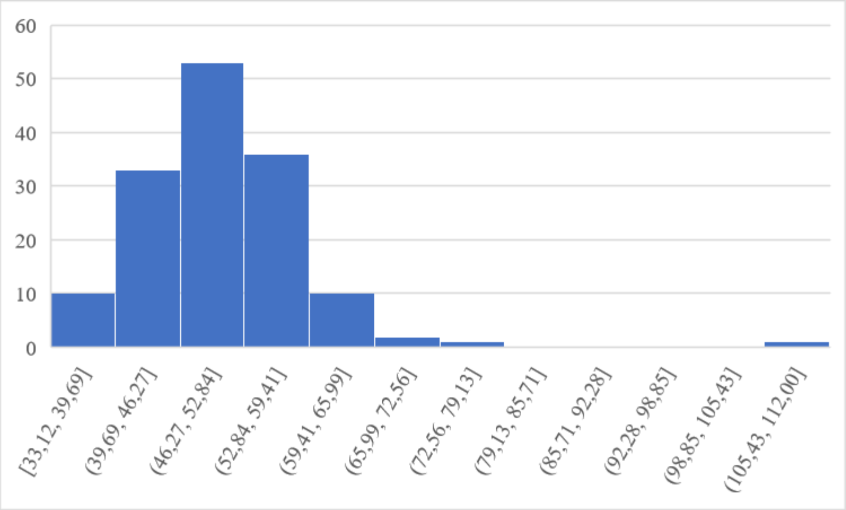


Figure 19: *Hypsodiametric ratio distribution in the pure plots.*

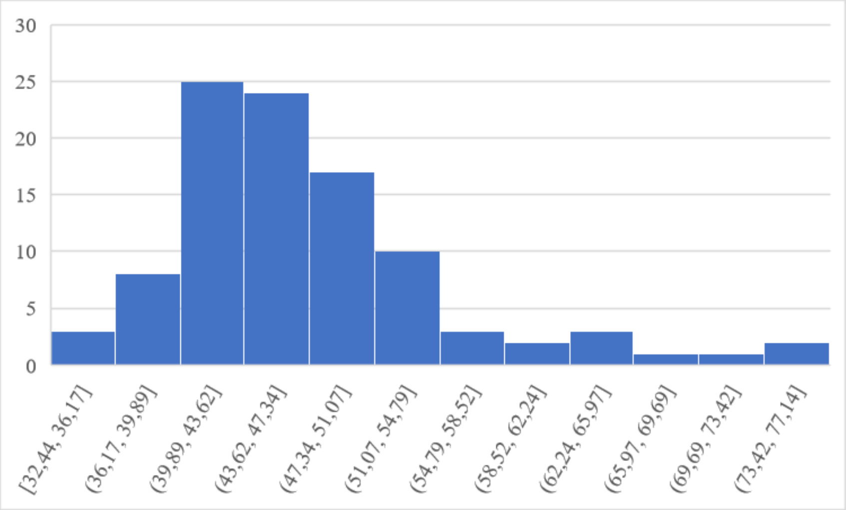


Figure 20: *Hypsodiametric ratio distribution in the mixed plots.*

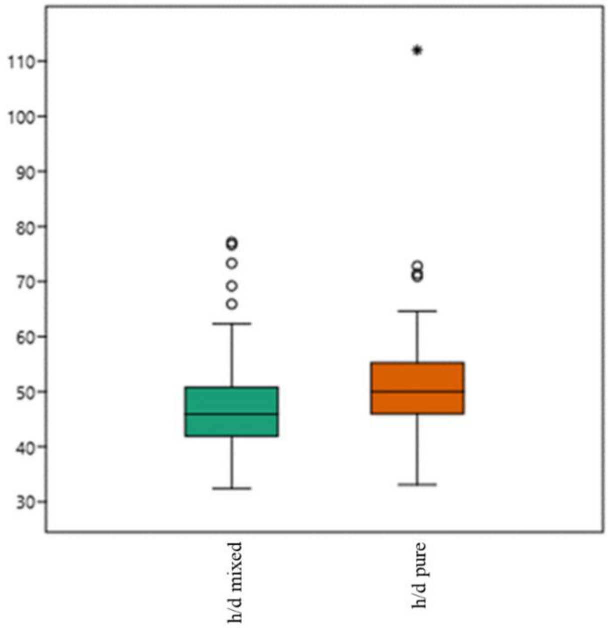


Figure 21: *Hypsodiametric ratio distribution in the two studied forest stands.*

Another aspect that transcends field sampling is that, in the pure stand, silver fir regeneration is sporadic and present only in the marginal areas or where, due to crashes, more light penetrates; while in the mixed stand, the regeneration of beech, a sciaphilous species in the juvenile stages of life, can be seen, which finds its optimal place to grow within the forest, taking advantage of the cover offered to it by silver fir (Bernetti, 1995).

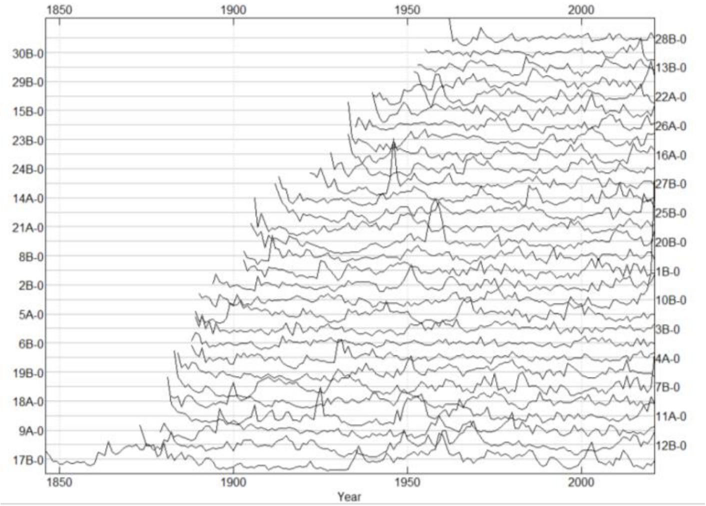


Figure 22: *Time series of silver fir tree growth for the mixed stand.*

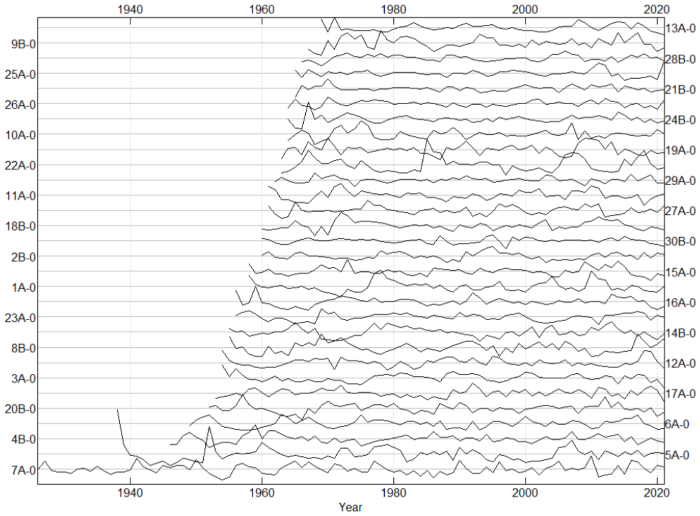


Figure 23: *Time series of silver fir tree growth for the pure stand.*

In Figures 22 and 23, shown above, it can be seen that the two stands have different ages, in particular the pure stand is younger than the mixed stand. Moreover, the mixed stand (Figure 19) has a typical uneven-aged structure, while the pure stand (Figure 20) is typically even-aged.

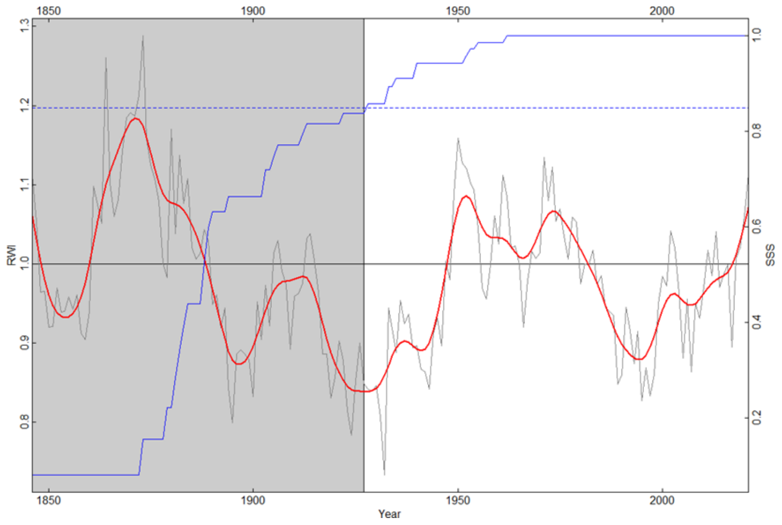


Figure 24: *Standardized chronologies of silver fir in the mixed stands.*

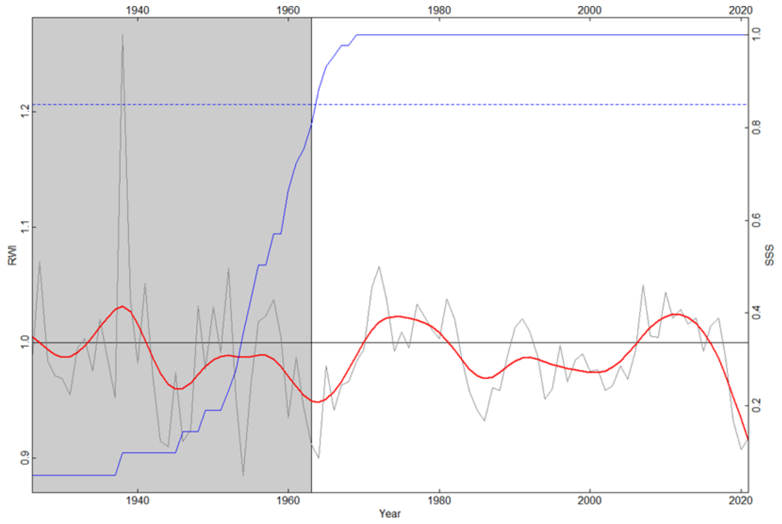


Figure 25: *Standardized chronologies of silver fir in the pure stands.*

Standardized chronologies for the pure and mixed stands of silver fir are shown in Figures 24 and 25. The figures show, in gray, the standardized Ring Width Index (RWI) values for the species, together with the version with attenuated extreme values obtained using a Spline curve (red line). In contrast, the blue line indicates the value of Subsample Signal Strength (SSS, Cook & Kairiukstis, 1990). The gray area indicates the portion of the chronology where SSS < 0.85, which is generally discarded from the analyses and would probably have needed additional samples. The useful portion of chronology begins around the 1970s for the pure stand, while it is around the 1930s for the mixed stand. After passing the critical threshold, however, growth was constant in the two situations. Comparing the two stands over the common period, the changes in growth are found to be smaller in the pure stand than in the mixed stand.

3.4. Discussion

Fungi infection may be limited by species mixing, thus reducing its propagation through trees; this is because the fungus also tends to spread through anastomoses, i.e. through root contacts (Asiegbu, 2000; Gaitnieks et al., 2021). As reported by other authors (Cherubini et al., 2002), our results demonstrate that abrupt growth reductions are good indicators of fungal attack. The absence of the fungi attack in the pure stand stress allows more regular stem growths. The lower incidence of *Heterobasidion* ssp. in silver fir in admixture with beech is probably because in mixed stands there is a wider spacing between susceptible trees, in this case silver fir, which will delay the spread between those individuals (Werner & Łakomy, 2002).

Additionally, in mixed stands there are probably fewer root contacts between the silver fir trees, as showed by the results obtained by the structural analyses. Root grafts between individuals of different tree species have been found to be less abundant than between individuals of the same species, as showed by (Lindén & Vollbrecht, 2002).

However, divergent results have also been reported, especially for central Europe. Several investigation could not demonstrate any clear correlation between the total fungi attack on silver fir and the proportion of admixed trees (Von Siepmann, 1988), where the mixing effect was obscured by other factors that had a stronger influence on the occurrence of the infection, such as thinning operation and mechanical damaged deriving from natural disturbances, such as strong winds.

However, comparing our data on with those from other similar mixed and pure forests is difficult because of the absence of similar studies, especially in our Mediterranean mountainous forest ecosystems, that compared the forest types characteristics and fungi occurrence. For this reason, additional data would be necessary to better understand the factors influencing the occurrence of the *Heterobasidion* ssp. in relation to the forest species composition and mixture.

Starting from the end of the eighteenth century, the frequent replacement of broadleaved tree species (especially beech) with conifers and the production of fresh food bases (stumps) could have created more favorable conditions for the fungi here studied. As modern and sustainable forest management should then be aimed to re-convert the monocultures into mixed broadleaved-conifers stands.

As already reported by recent studies (Marziliano et al. 2011; Russo et al. 2019), the results confirm that the mixed stand ensures a greater volume increment than that observed in the pure stand, thus testifying how the mixed forest constitutes a stable system, highly productive and capable of conserving the natural factors of production, at a high level. Silvicultural guidelines with regard to silver fir, should pursue the goal of its conservation because of its importance in phytogeographic, genetic and landscape terms. Based on the observed regeneration patterns, the application of low-impact interventions based on the observation of natural phenomena can be considered valid. In wetter exposures, a system of small hole cuts irregularly distributed in time and space could be an effective practice for silver fir regeneration in the southern Apennines (Mercurio & Mercurio, 2008). Therefore, management hypotheses should aim at reconstituting complex structures. To achieve this goal, it is not possible to resort to the application of classical forms of treatment; on the contrary, forest-supportive interventions, freed from any rigid and pre-constituted scheme, should be adopted according to the approach of systemic silviculture (Ciancio & Nocentini, 1996). It is then important to support thinning treatments aimed at leaving mixed stands. Forest ecosystems are, in fact, complex biological systems that result from the interaction between various factors, both natural and man-made (Nocentini, 2005). A key feature of them is that they are constantly changing, thus adapting to environmental factors (Meffe & Carroll, 1997), so from the results obtained from this study and the other studies cited above, trying to maintain stands in a mixed condition and promoting the gradual transition of pure stands into mixed stands is a necessary action to increase conservation, complexity and biodiversity, so as to promote a greater ecological stability.

3.5. Conclusions

As a result, it was found that the structure of the two situations (pure and mixed) is significantly different. In fact, the plants have a higher hypsodiametric ratio in the pure forest as well as a higher density, while in the mixed forest there are fewer and larger individuals.

Silver fir growing in mixed stands with beech appeared less interested by the occurrence of fungi attacks when compared with the pure silver fir stand. Fungi infection may be limited by species mixing, thus reducing its propagation through trees; this is because the fungus also tends to spread through anastomoses, i.e. through root contacts

Basing on the results obtained through the structural and dendrochronological analyses, it is confirmed that the growth variations are found to be smaller in the pure stand than in the mixed stand for silver fir. In addition, the mixed stand ensures greater volume increase than that observed in the pure stand, thus testifying to how the mixed forest contributes to constitute a stable, highly productive system capable of conserving natural production factors to the best of its ability, at a high level. Management hypotheses should therefore aim at the reconstitution of complex structures and it is then important to support thinning treatments aimed at leaving mixed stands. Forest ecosystems are, in fact, complex biological systems that result from the interaction between various factors, both natural and man-made. Basing on the results obtained in this study, we suggest to maintain stands in a mixed condition, promoting the gradual transition of pure stands into mixed stands, in order to increase the structural complexity and to promote the ecological stability within them.

CHAPTER 4

4. Wood quality of silver fir affected by *Heterobasidion* ssp. in pure and mixed forest stands in the "Serre" Regional Park (Southern Italy)

Abstract

The aim of this study was to verify whether the quality of the woody products and their biomechanical characteristics can be improved by promoting mixing between tree species, with particular reference to pure and mixed stands of beech *(Fagus sylvatica* L.) (FS) and silver fir (*Abies alba* Mill.). In particular, considering that silver fir growing in mixed stands is less affected by the presence and propagation of *Heterobasidion* spp. roots, it was hypothesized that silver fir woody materials are of better quality when derived from stands mixed with beech. In order to test this hypothesis, non-destructive methods (TreeSonic timer) were used to estimate the Dynamic Modulus of Elasticity (MOEd) and infrared spectroscopy (TF-IR spectrometer) was also applied to estimate the presence and abundance of molecular components – e.g. lignin and cellulose - as main determinant of the wood technological properties. For the above cited purposes, an LDA+clustering model was also applied. In addition, an attempt was done to construct a model based on linear discriminant analysis and developed in Python to distinguish different forest types on the basis of the IR spectrum. The results obtained highlighted the complexity of the interactions between the studied tree species in mixed stands, revealing that, although in mixed stands trees were smaller in diameters than in the pure formations, both silver fir and beech benefit from the mixture. Silver fir trees grown in the pure stand were characterized by a high incidence of *Heterobasidion* spp. infestation which affected the wood quality. Furthermore, a more regular tree growth trends were observed in the mixed stands with benefits for wood quality, also confirmed by the higher MOEd values recorded in the mixed plots, together with the higher levels of cellulose and lignin. Therefore, forest management that support mixed forests seems, for the species studied, to improve the wood quality and, consequently, the timber commercial value. Further studies will determine whether the general positive effects of mixing silver fir and beech can be generalized to other species of forest interest.

Keywords: Tree mixture, Tree Sonic, Dynamic Modulus of Elasticity (MOEd), Infrared spectroscopy, Wood molecular components, LDA and clustering model.

4.2. Introduction

Although mixed-species stands may overyield forest monocultures up to 30% (Bielak et al., 2014; Zhang et al., 2012), site conditions, stand age and tree species interactions affect these responses (Forrester, 2014; Petráš et al., 2016). Focusing on the causes of differences in tree growth and stand productivity between mixed-species and pure forests, most research addressed environmental settings and relationships with climatic conditions, species composition, mixture type, and stand age (Hausser & Troeger, 1967; Kennel, 1966; H. Kramer, 1988; Magin, 1954; Mettin, 1985; Mitscherlich, 1967; Pretzsch et al., 2010). Indeed, few studies exist on the quality and value of wood produced in mixed-species vs. the corresponding pure stands (Saha et al., 2012). Information on how wood quality can be affected in relation to tree species composition is essential for decision making in adaptive forestry, especially where forest planning and thinning activities support the occurrence of mixed-species stands (Petráš et al., 2016).

Liu et al. (2018) showed that spacing and thinning experiments in pure stands highlighted the strong effect of the surrounding spatial stand structure on tree growth and morphology and, ultimately, on the wood structure and timber quality. Several studies found that mixed-species plantations might be more productive in comparison with monocultures (Kanowski et al. 2005; Petit & Montagnini 2006; Pretzsch & Rais 2016; Richards et al. 2010; Zhang et al. 2012). Chomel et al. (2014) demonstrated that mixing hybrid poplar and white spruce might increase wood production of poplar in comparison with monocultures of either poplar or white spruce. Battipaglia et al. (2017) showed a considerable increase in cumulative basal area and in intrinsic water use efficiency in mixed-species stands of pedunculate oak and Italian alder, largely resulting from an increase in N fixation, which levelled off, when natural mortality or management practices decreased the competitive ability of Italian alder. In addition to stand productivity and water use efficiency, species mixtures with structural stratification may also enhance individual-tree growth rates and stem quality of species in the upper canopies, minimizing the proportion of taller species that reach the highest production (Kelty, 2006; Piotto, 2008).

Forest management practices oriented to obtain mixed-species stands potentially determine variation in the physical and mechanical properties of the harvested wood, both at stand and tree level (Machado et al., 2014). Nevertheless, though mixed-species forestry is gaining popularity in Europe (Pretzsch & Schütze 2009, 2016), reporting differences in wood quality between mixed-species vs. corresponding pure stands is needed. In the last decades, the evaluation of wood quality has been preferentially based on non-destructive technologies (NDTs) (Russo et al., 2019). More specifically, stress wave-based non-destructive acoustic techniques resulted in very useful methods for predicting the mechanical properties of woody materials (Guntekin et al. 2013). Nowadays, the acoustic sensing technology allows the estimation of wood quality and intrinsic woody properties for standing trees, stems and logs. Among the parameters measurable by acoustic methods, the most important are the modulus of elasticity of wood (MOE) and the dynamic modulus of elasticity (MOEd), being related to wood anatomy and tree physiology. These parameters are fundamental for the evaluation of wood quality, providing information on the resistance to deflection and the stiffness of material (Teder et al. 2011; Wessels et al. 2011).

In a mountainous-Mediterranean context of the southern Apennines, we aimed to verify how the quality of woody products and their biomechanical characteristics can be improved by the promotion of mixing between tree species, with particular reference to pure and mixed forests of beech (*Fagus sylvatica L.*) (FS) and silver fir (*Abies alba* Mill.) (AA) found in the “Serre” Regional Park (Stilo-Archiforo Forest). In detail, a stand with mixed specific composition (FS - AA) and, as a control, two single-species dominated forest areas (FS and AA) were identified, subjected to ordinary forest planning and occurring under similar environmental, climatic and ecological conditions. Assuming that mixed forest can improve the quality of the wood produced, triplets of circular test plots of 20 m radius were sampled in each of the three selected conditions.

The aim of this study was to verify whether the quality of the woody products and their biomechanical characteristics can be improved by promoting mixing between tree species, with particular reference to pure and mixed stands of beech *(Fagus sylvatica* L.) (FS) and silver fir (*Abies alba* Mill.). In particular, considering that silver fir growing in mixed stands is less affected by the presence and propagation of *Heterobasidion* spp. roots, it was hypothesized that silver fir woody materials are of better quality when derived from stands mixed with beech (Pukkala, 2017). To test this hypothesis, non-destructive methods (TreeSonic timer) were used to estimate the Dynamic Modulus of Elasticity (MOEd) and infrared spectroscopy (TF-IR spectrometer) was used to estimate the presence and abundance of molecular components – e.g. lignin and cellulose - as main determinant of the wood technological properties. For the above cited purposes, an LDA + clustering model was also applied. In addition, an attempt will be made to construct a model based on linear discriminant analysis and developed in Python to distinguish different forest types on the basis of the IR spectrum.

For the latter aspect, non-destructive methods (TreeSonic timer) useful for estimating the Dynamic Modulus of Elasticity (MOEd) and infrared spectroscopy (TF-IR spectrometer), useful for estimating the presence and abundance omolecular components such as lignin and cellulose, were also used.

4.3. Materials and methods

4.3.1. *Study area and tree sampling*

Field activities were carried out in 2021. The studied site is located in Southern Italy (“Serre” Regional Park) in the "Bosco di Stilo-Archiforo", SCI IT9350121 – Calabrian Region (Figure 26).

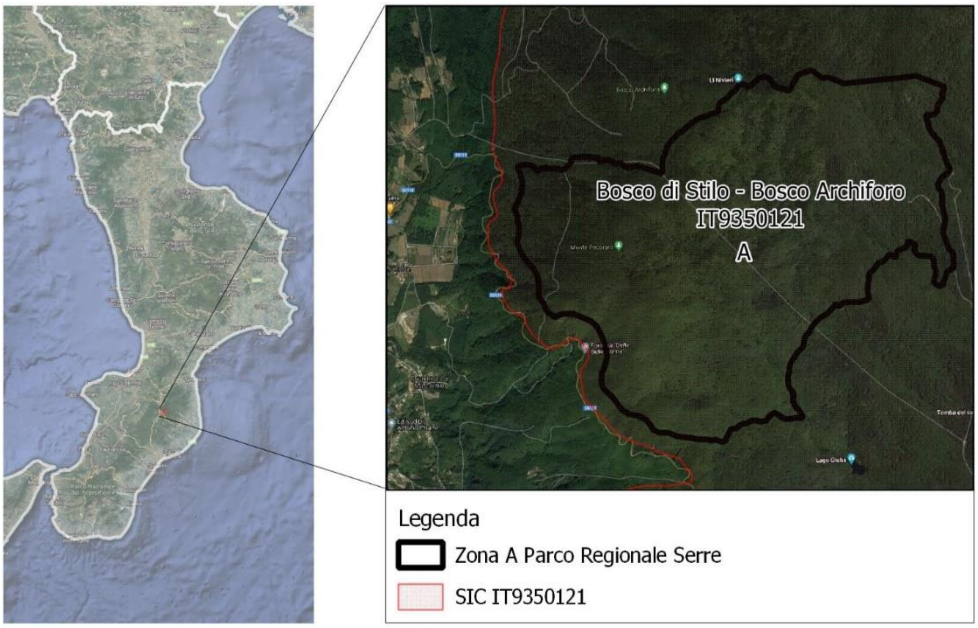


Figure 26: *Location of the study area in the context of the “Serre” Regional Park (Calabria Region).*

The survey protocol applied for the analysis of structural data and tree wood sampling in the forest involved the use of a completely randomized block design (CRBD). Using this design, three different theses were distinguished: pure silver-fir forest, pure beech forest, and mixed silver fir-beech forest. The field activities were divided into subgroups called blocks, such that the variability within blocks (internal test variability) was less than the variability between blocks (due to environmental conditions or other factors outside the experiment area). This randomized block design removes a potential source of error by reducing the variability of external effects, then producing a good estimate of treatment effects when each treatment has an equal proportion across blocks (Table 2).

Table 2: *Sampling approach applied in the field - Completely Randomised Block Design Scheme (CRBD).*

	Treatment 1	Treatment 2	Treatment 3
Block 1	**Fs**	**AF***	**Aa***
Block 2	**AF***	**Aa***	**Fs**
Block 3	**Aa***	**Fs**	**AF***

Note: **Fs**: *Fagus sylvatica* L. pure; **Aa**: *Abies alba* Mill. pure; **AF**: Mixed stand *Fagus sylvatica L./Abies alba* Mill.; the * simbol represents the Block/Treatment wher fruit bodies were collected and analyzed.

Each triplet consists of three circular test areas, for silver fir pure forest, beech pure forest and mixed stands of the previous species. The size of the test circular area was 0.12 ha (20 m radius). The areas were chosen trying to minimize the stationary differences between each stand (exposure, elevation, soil fertility, etc.) and to have a minimum number of trees of 20 for the pure stands and 20 for each species in the mixed stand.

The areas were considered as pure stand when the basal area of the studied tree species represented at least the 85% of the total. Mixed species stands, on the other hand, were selected when the least represented of the two species contributed at least for the 20% of the basal area. The areas studied have similar exposure (north, northwest) and slope ranging between 0° and 20°. The main soil type was, according to FAO classification, Dystric Leptosol.

For each tree falling inside the plots, the diameter at 0.30 m and 1.30 m above ground level (DBH) was measured, while each tree height was measured with a Suunto hypsometer; similarly, crown insertions height were also recorded. Two cores per tree were also extracted through the use of a Pressler borer at 1.30 m above the ground. Cores were extracted at 120° each other to the direction of maximum slope, in order to avoid tension/compression wood sampling, if any. In total, 180 trees were sampled for a total of 360 cores.

4.3.2. Estimation of the Modulus of elasticity (MOEd).

A sample of 100 trees was selected for each thesis described above, on which measurements of dynamic modulus of elasticity (MOEd), a parameter closely related to overall wood quality, were made. A TreeSonic™ FAKOPP (Fakopp Enterprise, Agfalva, Hungary), a patented instrument for measurement on standing trees (Wang, 2011, 2013) , was used for measurement. The TreeSonic consists of two acoustic piezosensors (emission sensor and receiver), a two-channel data-logger, and a hammer (Figure 27).

Figure 27: *(a) TreeSonic™ ultrasound instrument and (b) Operating diagram from* (Proto et al., 2017)**.**

During the field measurements, sensors were inserted into the trunk, aligned in a vertical plane and angled toward each other. The lower probe was placed about 80 cm above the ground, while the distance between the upper and lower sensors was 1.0 m. When the "start" sensor was hit with the hammer, it generated a sound wave that propagated inside the tree and then received by the lower sensor, while the data-logger showed the time of travel of the wave (TOF, Time Of Flight) in microseconds. This value, together with the distance (D) between the sensors was used to determine the speed of the sound wave (Equation 1):

$$AV = D \cdot TOF^{-1} \text{ [m s}^{-1}\text{]} \qquad (1)$$

Finally, the MOEd value was obtained by multiplying the value of the sound wave velocity by the value of the wood density, WD (Equation 2):

$$MOEd = WD \cdot AV^2 \text{ [kg m}^{-1}\text{ s}^{-2}\text{ = Pa = N m}^{-2}\text{]} \qquad (2)$$

4.3.3. Dendrochronological analyses

The cores were prepared following well-established dendrochronological methods (Fritts, 2012): specimens were dried, glued on wooden supports and then sanded with a belt sander to make annual accretions more visible. Ring width was measured to an accuracy of 0.01 mm using a Leica MS5 stereoscope (Leica Microsystems, Wetzlar, Germany) connected to a LINTAB measuring device (Rinntech, Heidelberg, Germany). The "mean tree" was derived by cross-dating the two cores extracted from each plant using the TSAPWIN software package (version 4.81c) and choosing the cross-dating with the highest GLK (Huber, 1943) index value and, in any case, greater than 0.75. These measurements were then verified using the Dendrochronology Program Library in R (dplR) package (Bunn et al., 2010), calculating statistics and checking the correlation of each tree with the main chronology. After validation of the data, the decreasing trend due to the tree aging was removed from each ring series with an AR1 model, and the derived series were standardized with a spline curve (20-year window and 10-year overlap) to obtain the average stand chronologies, developed for each species using Tukey's robust average, which reduces the sensitivity of the calculation to outliers.

4.3.4. TF-IR spectrometer analyses

For each plot, 3 cores were selected to characterize their chemical composition with a TF-IR spectrometer. For each sample, in order to avoid the initial growth period, often characterized by strong competition between trees, the period of the last 10 years and the previous 10 years (considering that the age is on average greater than 70 years) was used. Each of the samples was reduced to a powder of uniform size ($d \cong 0.05$ mm) and analyzed with a Nicolet iS50 FT-IR spectrometer (Thermo Fisher Scientific Co., Waltham, MA, USA) at the laboratories of "La Yutera" Campus (Palencia, Spain). The instrument has a spectral resolution of 0.482 cm^{-1} (wavenumber range of 4 cm-1) with a spectrum analysis range of 400 to 4000 cm^{-1}. The spectrum of each sample was calculated as the average of 64 following scans in absorbance mode. The near-infrared (NIR) spectra of wood contain information about their chemical composition and molecular structure. Both influence the physical properties of the material; however, at present, this information is generally underutilized. In this study, IR spectroscopy was mainly used in a qualitative-exploratory approach, determining the proportion of the main chemical constituents (lignin and cellulose) that influence the physicochemical and mechanical properties of the woody materials. The data obtained were analyzed using scripts made ad hoc in Python (Van Rossum & Drake, 2000) while Past software (Hammer et al., 2001) was used for statistical analysis. The spectrum analysis was limited to the area with wavenumber <= 1800 (fingerprint area), since the area between 4000 and 1800 WN (functional group area) contains no distinguishing features for the substances of interest (Wade, 2006). Finally, average spectra in the fingerprint area for each forest type were calculated and the peak absorbance identification was conducted.

4.3.5. Statistical analysis and ML model

Given the good differentiation between the IR spectra for the samples examined, they were used to discriminate forest type by means of a simple model built ad hoc in Python, using the scikit-learn library (Pedregosa et al., 2011). The model is a pipeline consisting of three steps:

- Pre-processing: Standardization of raw wavenumbers (72 samples with 2907 absorbance measurements each along the IR spectrum);

- Dimensionality reduction: using Linear Discriminant Analysis (LDA), axes are derived that maximize the separation between classes, projecting the data into a lower dimensionality space. In general, dimensionality reduction helps reduce computational costs for classification tasks, and is useful for avoiding overfitting by minimizing error in parameter estimation:

- Clustering: (K-Means algorithm) of LDA output components in the 4 forest types.

Linear Discriminant Analysis (LDA) is a generalization of Fisher's linear discriminant, a method used to find a linear combination of variables that characterizes or separates two or more classes of objects. The resulting combination can be used as a linear classifier or, more commonly, for dimensionality reduction before subsequent classification. LDA is closely related to analysis of variance (ANOVA) and linear regression, as well as to principal component analysis (PCA) and factor analysis, but unlike the latter, LDA arose explicitly to model differences between classes of data. It is used when groups are known a priori.

4.4. Results

4.4.1. Forest structural features

The analysis of the data collected in the field, summarized in Table 3, shows the different response of the two studied species when growing in pure or mixed forests. Silver fir trees grown in the pure stand have both greater diameters and heights overall than silver fir grown in the mixed stand. It should be noted, however, a high incidence of *Heterobasidion* spp. infestation (on about the 80% of the trees) which impairs the final quality of the wood by causing rot; silver fir regeneration is also sporadic and present only in the edge areas or where, due to canopy gaps, more light penetrates. In the mixed stand, on the other hand, probably due to the interruption of the root continuity between plants, the fungus is almost completely absent. Also for beech, slightly smaller diameters but significantly greater heights were found in the mixed forest; crown insertion height was generally higher than in the pure forest, reducing the probability of knot formation. From a phytosanitary point of view, no particular emergencies were observed, whereas in pure beech forest,minor infestations of defoliating lepidoptera occurred.

Table 3: *Summary of the structural features of the studied stands.*

Forest type	**D (average) cm**	**H (average) m**	**N. trees n.**	**G (Basal area)** *m^2 ha^{-1}*	**H/D ratio**
Pure Silver fir	52.5	31.3	318	68.5	59.5
Silver fir in mixed stand	47.3	25.9	133	23.0	54.7
Pure Beech	38.2	20.9	252	28.6	54.9
Beech in mixed stand	33.2	24.9	135	11.5	75.9

The hypsometric curves of the stands revealed that the silver fir growth appears to be little affected by the mixing effect, while beech trees growing in mixture with silver fir grows more in height in the smallest diameters and, in any case, always showing higher heights than in the pure forest (Figure 28).

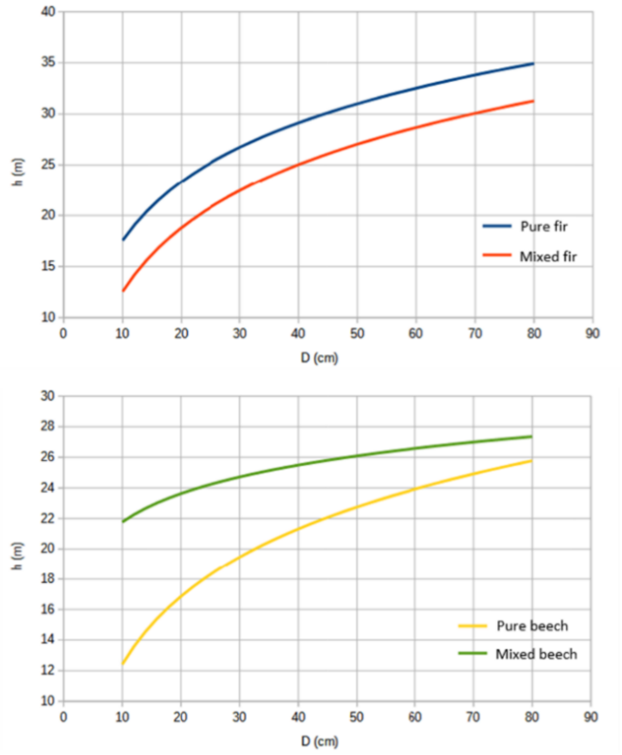


Figure 28: *Hypsometric curves of the studied species in the pure and mixed stands..*

4.4.2. Estimation of the Modulus of Elasticity (MOEd)

Processing of the data obtained from TreeSonic showed that, in mixed forests, the MOEd values were on average higher than in pure stands for both the examined species (Figure 29 a, b). Silver fir had an average value of 7630 N m^{-2} in pure stands and about 9570 N m^{-2} when growing in mixture with beech. Similarly, an average MOEd of 8140 N m^{-2} was found in pure beech forest, increasing to 10445 N m^{-2} in the mixed forest. The differences were significantly different (Kruskal-Wallis test and Mann-Whitney post-hoc, H(chi2) = 137.8, $p << 0.01$). As for silver fir, this finding was in agreement with the observations of the presence of *Heterobasidion* spp. in the pure forest, which degrades the wood and also affect significantly the speed of sound wave transmission.

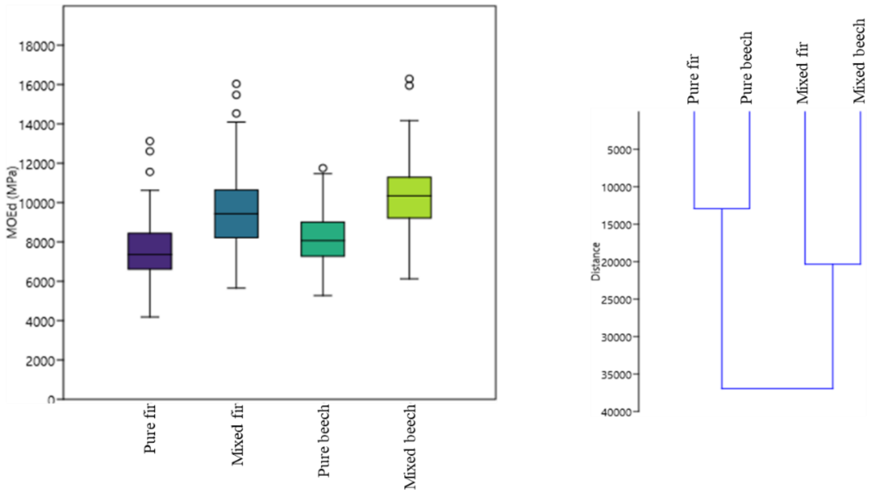
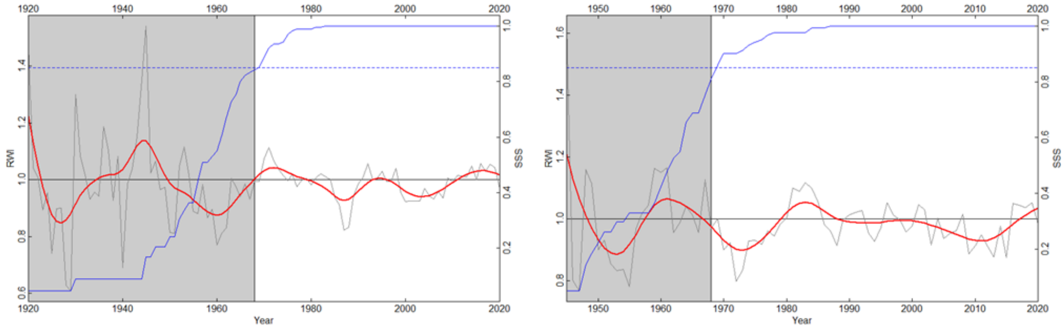


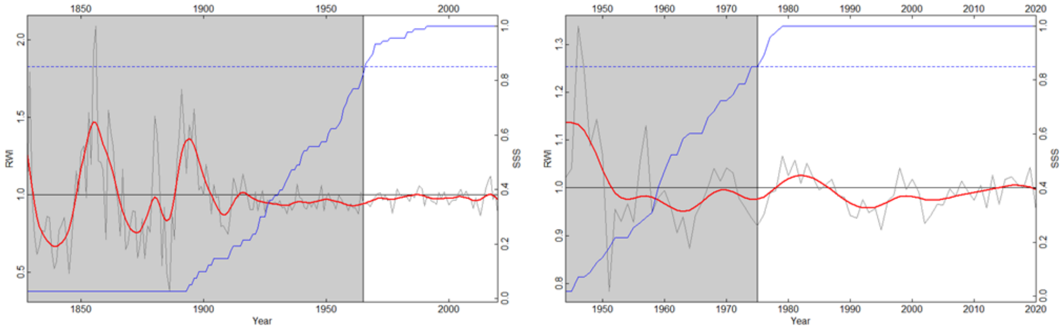
Figure 29: *MOEd values obtained for each species occurring in the mixed and pure stands; (a) box-plot of distributions and (b) cluster analysis.*

4.4.3. Dendrochronological traits

The dendrochronological analyses gave the site history for each forest type. Figure 30 shows, in gray, the standardized Ring Width Index (RWI) values for the species, together with the version with attenuated extreme values obtained using a Spline curve (red line). In contrast, the blue line indicates the Subsample Signal Strength (SSS; Cook & Kairiukstis, 1990) value. The gray area indicates the portion of the history where SSS < 0.85, which is generally discarded from the analyses. The useful portion of the chronology begins around the 1970s for each of the chronologies.



(a) (b)



(c) (d)

Figure 30: *Standardised chronologies for (a) pure silver fir forest, (b) mixed silver fir forest, (c) pure beech forest and (d) mixed beech forest (RWI: Ring Width Index, SSS: Subsample Signal Strength).*

4.4.4. TF-IR model data

From the analysis of the samples at the spectrophotometer, absorbance spectra were obtained, and it was verified that dividing each sample into two sub-samples (last 10 years and previous 10 years) did not result in statistically significant differences between the two periods (Anderson-Darling test, Z: 0.063, p: 0.309). The spectra (Figure 31a) were then averaged, for each forest type, to obtain the average spectrum and identify the presence of infrared absorbance peaks (Figure 31b).

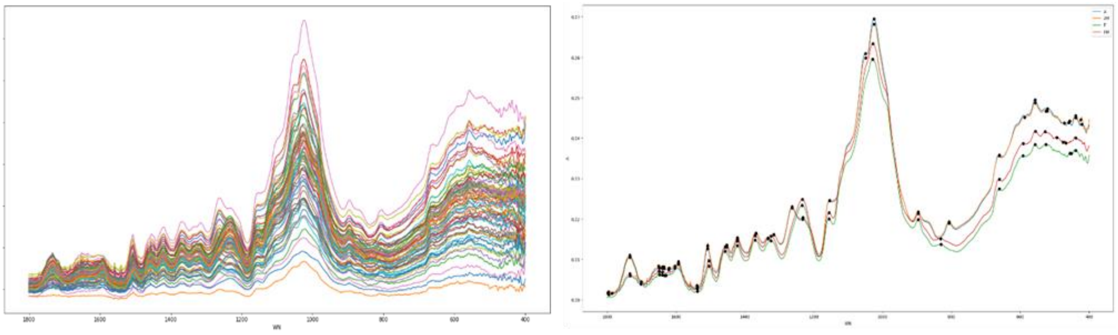


Figure 31: *(a) Infrared spectra of the samples and (b) average spectra per forest type with an indication of the recognised absorbance peaks (FT-IR spectrometer Nicolet iS50 Thermo Fisher Scientific Co).*

Through analysis of the absorbance intensity corresponding to certain wavenumbers, it was possible to determine the ratios between the lignin content and the various cellulose fractions; in particular, the following wavenumber ratios were used:

- 1104/1508 cm^{-1} to identify the holocellulose/lignin ratio
- 1104/1732 cm^{-1} for the holocellulose/hemicellulose ratio
- 1732/1508 cm^{-1} for the hemicellulose/lignin ratio
- 1056/1508 cm^{-1} for the cellulose/lignin ratio

It was observed that, in the mixed forest, the range of variation is always larger than in the pure forest (Figure 32).

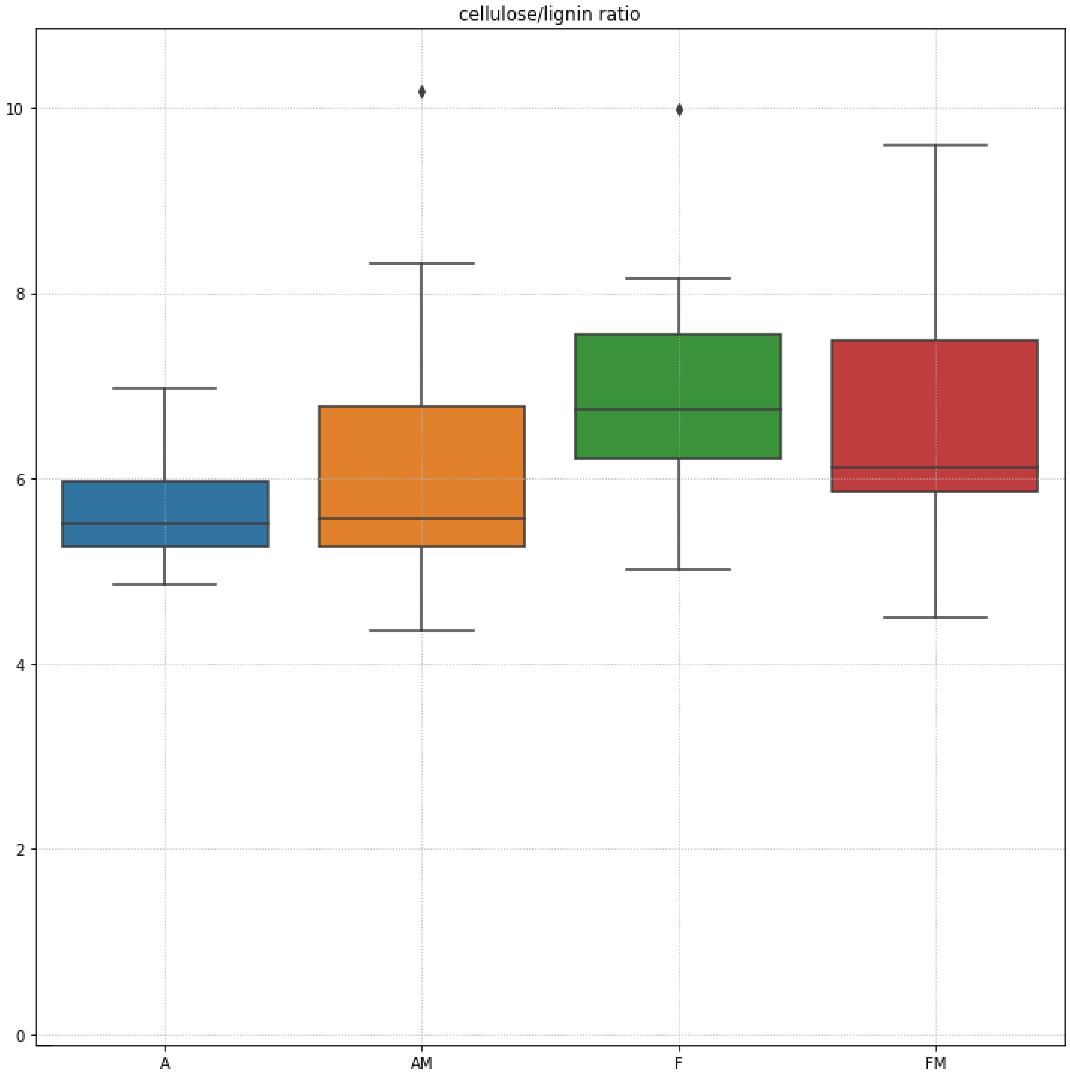
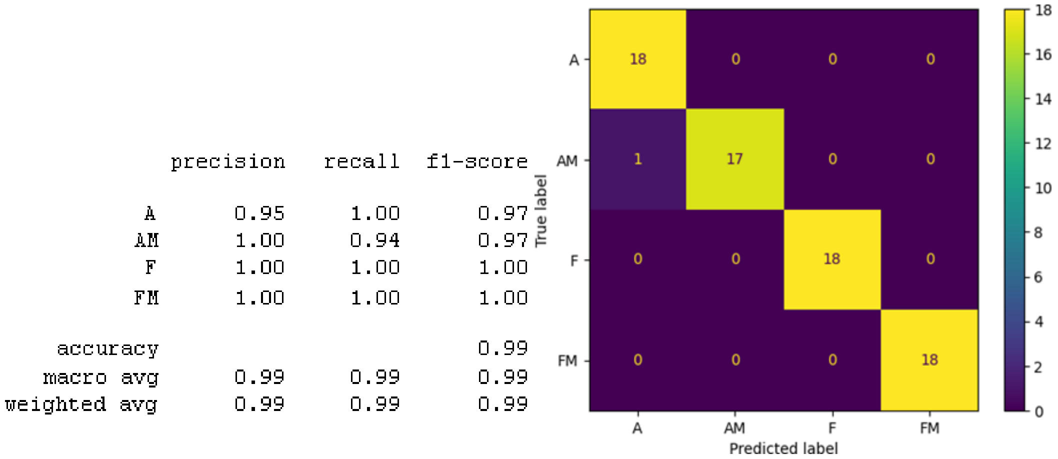


Figure 32: *Wavenumber ratio for pulp/lignin, by forest type. (A: pure silver fir; AM: silver fir in the mixed stand; F: pure beech; FM: beech in the mixed stand).*

4.4.5. ML model

The results obtained from the LDA+clustering model (Figures 33 and 34) showed a good discriminatory ability of the "fingerprint area" of the IR spectrum in separating forest types. In particular, the confusion matrix (Figure 30b) underlines that only one false negative (lower left part of the matrix) and no false positive (upper right part of the matrix) was predicted. The overall classification accuracy was 99%. This result also follows from the different proportion of macro-components (mostly lignin and cellulose) between pure and mixed forest, even within the same species. Considering that particular care was used during the identification of the study area to minimize the stationary differences between sites, the different composition in lignin/cellulose is likely to be attributed, in the mixed forest, to interactions between plants of different species.



```
              precision    recall  f1-score

           A       0.95      1.00      0.97
          AM       1.00      0.94      0.97
           F       1.00      1.00      1.00
          FM       1.00      1.00      1.00

    accuracy                           0.99
   macro avg       0.99      0.99      0.99
weighted avg       0.99      0.99      0.99
```

Figure 33: *(a) Classification report and (b) confusion matrix of the LDA model.*

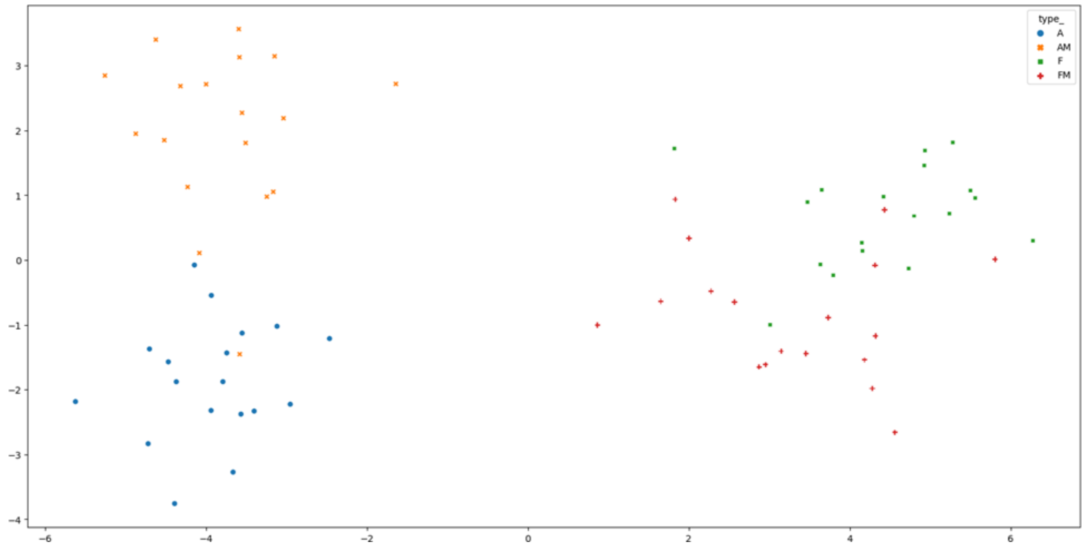


Figure 34: *Graphical output of the LDA model (A: pure silver fir; AM: silver fir in the mixed stand; F: pure beech; FM: beech in the mixed stand).*

4.5. Discussion

Results demonstrated how the mixture effect influenced both wood quality and tree ring widths. The greatest advantages for the silver fir are those related to its phytosanitary status: in the pure silver fir forest, in fact, most of the trees are affected to varying degrees of fungal infestations, especially of the genus *Heterobasidion* spp, as demonstrated above. This pathogen causes the trunk rot in the first few meters of the tree's height, leading to stability problems and, from a commercial point of view, causes the loss of the most valuable part of the stem for further processing. In mixed forests, on the other hand, the presence of these pathogens was almost negligible. The lower presence of this stress allowed a more regular growth of the stem and its exploitation for commercial purposes.

Under pure conditions, beech trees showed the insertion of the first branches at significantly lower heights than beech trees grown in mixed forests. The high presences of branches increases the probability of wood defects related to fibers continuity, generating knots (live and/or dead) and interrupting the uniformity of the wood's mechanical characteristics. This phenomenon is greatly attenuated in mixed forests, where the crown insertion height is generally higher than 5 meters.

In the mixed forest, moreover, competition for sunlight leads the beech to develop more in height, allowing longer woody assortments.

A combination of a light demanding tree species with one more shade tolerant was probably at the base of these results. In other studies, mixed-species stands were found to show higher light interception and light-use efficiency in comparison with monocultures (Binkley et al., 1992; Forrester et al., 2012; Kelty, 1992; Pretzsch & Schütze, 2016). Promoting the coexistence of these two species, at least in the studied environmental conditions, might improve the quality of woody materials, regardless of the tree size from which the assortments are obtained.

The MOEd values, strongly related to the wood density and acoustic velocity, indicated an overall higher mechanical stability attained in the mixed-species stands. In fact, wood density is correlated with timber strength (Saranpää, 2003), hardness and abrasiveness (Bacher & Krzosek, 2014) . Therefore, the species mixture might provide higher mechanical resistance to natural disturbances, such as windstorms.. However, it must be pointed out that (Pretzsch & Rais 2016) observed that the wood strength and stiffness could be lower in complex forests than in homogeneous monocultures, where tree size and shape development progress more continuously. Yet, Torquato et al. (2014), in black spruce forests in Canada, detected lower strength and stiffness properties in complex stands.

Admixture of beech with silver fir might, therefore, reduce competition or even increase facilitation processes in harsh environments, such as those of Mediterranean mountains (xeric conditions). Beneficial effects on beech from admixtures were also reported in other studies (Pretzsch et al., 2013; Pretzsch et al., 2010). Again, Condés et al. (2013) reported that productivity of pine-beech admixtures was generally greater than in the corresponding pure stands.

However, the complexity of the species interactions, which depends on stand development stage, stand density and site conditions (Forrester, 2014), suggests caution in generalizing these results, since other studies reported opposite patterns (Zeller et al., 2017). Equal productivity at the stand level might not necessarily indicate a neutral behavior of the two species co-occurring in the mixed-species stands. In fact, species-specific reactions, at individual or stand level, might counteract and cancel each other with respect to the stand level productivity (Pretzsch et al., 2010). Behind overyielding or underyielding of mixed-species in comparison with nearby pure stands, as revealed in this study for silver fir and beech, there is always a modified supply and uptake, or different use-efficiency, of available resources (Binkley et al 2004; Forrester, 2014; Richards et al., 2010). Zhang et al. (2012) reported that beneficial effects of admixing provided an overall 25% increase in productivity across forest types and a 12% increase at European scale in Scots pine-beech mixtures e con (Pretzsch & Schütze, 2016). Nevertheless, the mechanisms that might promote complementarity effects, leading to increased productivity in pine-beech mixtures, are poorly understood, despite the frequent occurrence and economic importance of these forest types (Pretzsch & Schütze, 2016).

Structural and dendrochronological analyses showed a more regular growth in mixed situations than in pure forests, and the benefits on wood quality were confirmed by the higher MOEd values in mixed plots, together with the higher levels of cellulose and lignin in beech mixed with silver fir detected by TF-IR spectroscopy. It was also possible to construct a model based on linear discriminant analysis and developed in Python to distinguish different forest types based on the IR spectrum. The results obtained suggested, therefore, that the promotion of mixed forest in this environment could improve growth dynamics and wood quality in beech more than in silver fir. The better wood quality in mixed forest conditions, probably induced by the effects mentioned above, is further confirmed by the values obtained in the estimation of the modulus of elasticity, which were higher in the mixed forest, indicating a greater homogeneity of the woody materials.

4.6. Conclusions

The objective of this research was to verify the effects of forest management and specific composition in terms of qualitative and quantitative characteristics of woody products in silver fir and beech forests. The analysis of the available data highlighted the complexity of the interactions between forest species in mixed forests and showed that, although trees with slightly smaller diameters than in pure formations were generally found, especially for beech, however both silver fir and beech benefit from the mixture regime. Therefore, management oriented towards the promotion of mixed forests seems, for the species studied, to better support the tree growth and, consequently, to obtain timber of greater commercial value. Further studies will determine whether the general positive effects of mixing silver fir and beech can be generalized to other species of forest interest.

CHAPTER 5

Conclusions and future perspectives

The aim of this Ph.D. project was to investigate whether the presence of pathogenic conifer root rot fungi, such as *Heterobasidion* spp., can influence the productivity, the vitality and the growth trends of beech and silver fir forests, but also the wood quality of the materials harvested from them. Specifically, the research work was based on the hypothesis that mixed forests of beech and silver fir (*Abies alba* Mill. - *Fagus sylvatica* L.) may be better protected from root rot pathogens than the pure silver fir stands, and in particular from *Heterobasidion* spp., which mainly causes damage to conifers. By means of dendrometric analyses and dendrochronological approaches, it was tested whether the mixing of silver fir and beech species could improve the growth and fitness of silver fir, when compared to pure silver fir stands. It was also verified whether the quality of the woody products and their biomechanical characteristics can be improved by promoting the mixture between tree species. Non-destructive methods (TreeSonic timer), useful to estimate the Dynamic Modulus of Elasticity (MOEd) and infrared spectroscopy (TF-IR spectrometer), applied to estimate the presence and abundance of molecular components such as lignin and cellulose, were used. Moreover, a LDA + clustering model was also applied.

On the basis of the results obtained, the presence of the pathogen *Heterobasidion* spp., and in particular the intersterile group F (*H. abietinum*), was ascertained, and it was found that the tree growth variations were smaller in the pure stand than in the mixed stand for silver fir. Furthermore, the mixed stands ensured a greater volume increase for silver fir than in the pure stands, thus demonstrating that the mixed forest contributes to a more stable system, which also ensures greater productivity. In addition, the structural and dendrochronological analyses revealed a more regular tree growth trends in the mixed stands than in the pure ones. Furthermore, benefits on wood quality were confirmed by the higher MOEd values observed in the mixed plots, together with the higher levels of cellulose and lignin in the silver fir-beech mixed.

The results obtained revealed also that that the promotion of mixed forests in mountainous forest ecosystems could improve the tree growth dynamics and wood quality of beech more than of silver fir.

In addition, the mixed stand ensures a greater volume increase than that observed in the pure stand, thus demonstrating that the mixed forest contributes to a stable, highly productive system that is capable of conserving natural production factors to the best of its ability and acts as a filter and as a barrier to fungal infestations that propagate by anastomosis.

This study provided also interesting information useful to highlight the importance of the non-destructive methods for the evaluation of the wood quality. The benefits on wood quality were confirmed by the MOEd values recorded, together with the levels of cellulose and lignin in beech mixed with silver fir detected by TF-IR spectroscopy. The results, therefore, suggested that the promotion of mixed forest, at least in this studied environment, could improve the tree growth dynamics and wood quality of beech more than of silver fir. The better wood quality in mixed forest conditions, probably due to the above-mentioned effects, was further confirmed by the values obtained in the estimation of the modulus of elasticity, which were higher in the mixed forest, indicating a greater homogeneity of the woody material.

In conclusions, we can here adifirm that mixed-species stands of beech and silver fir in Southern Italy might represent a positive example of forest structures useful for improving the overall wood quality of both the species, at least in Mediterranean mountainous forest ecosystems. It could be interesting deeply verify if, in these harsh environments, the mixture could also increase the overall forest stand stability and the efficiency in the resources use.

Management hypotheses should therefore be aimed at the reconstitution of complex structures. A fundamental characteristic of forest ecosystems is that they are constantly evolving, thus adapting to environmental factors. Therefore, from the results obtained in this study and from the other studies mentioned above, trying to maintain stands in a mixed condition and promoting the gradual transition of pure stands into mixed ones is a necessary action to increase conservation, complexity and biodiversity, so as to fprovote the ecological stability within them.

Further investigation could be planned in the future, focusing also on other tree species, typical of the mountainous Mediterranean forest ecosystems. These approaches might permit the implementatation of more accurate models, useful to further improve the woody supply chain and the related economical incomes in the inner areas, promoting the mixture of species in order the reduce the risk of pathogens spread that can strongly affect the tree vitality, especially in a global change scenario.

Finally, during the Ph.D. course, the candidate was involved also in another research activity, realized in the context of the “Aspromonte” National Park. The main objective of the study was to determine the most suitable duration (in years) of cutting cycles to maximize stand productivity and preserve wood quality. More specifically, a growth model was developed to verify if wood quality of chestnut coppices at different stand ages varies when the rotation period is modified. Wood quality and stand productivity were analysed, using a chronosequence approach, in stands characterized by four cutting cycles (15, 25, 30, and 50 years). The study was published as peer-reviewed paper in 2022 on the Journal “European Journal of Forest Research” (Marziliano et al., 2022). The whole manuscript is reported at the end of this thesis, in the Appendix 1.

References

Altieri, V., De Franco, S., Lombardi, F., Marziliano, P. A., Menguzzato, G., & Porto, P. (2018). The role of silvicultural systems and forest types in preventing soil erosion processes in mountain forests: A methodological approach using cesium-137 measurements. *Journal of Soils and Sediments*, *18*(12), 3378–3387.

Asiegbu, F. O. (2000). Adhesion and development of the root rot fungus (Heterobasidion annosum) on conifer tissues: Effects of spore and host surface constituents. *Fems microbiology ecology*, *33*(2), 101–110.

Asiegbu, F. O., Adomas, A., & Stenlid, J. A. N. (2005). Conifer root and butt rot caused by Heterobasidion annosum (Fr.) Bref. Sl. *Molecular plant pathology*, *6*(4), 395–409.

Azeem, M., Barba-Aliaga, M., Borg-Karlson, A. K., Terenius, O., Broberg, A., & Rajarao, G. K. (2019). Heterobasidion-growth inhibiting Bacillus subtilis A18 exhibits medium-and age-dependent production of lipopeptides. *Microbiological research*, *223*, 129–136.

Bacher, M., & Krzosek, S. (2014). Bending and tension strength classes in European standards. *Annals of Warsaw University of Life Sciences-SGGW. Forestry and Wood Technology*, *88*.

Baillie, M. G., & Pilcher, J. R. (1973). *A simple crossdating program for tree-ring research*.

Battipaglia, G., Pelleri, F., Lombardi, F., Altieri, S., Vitone, A., Conte, E., & Tognetti, R. (2017). Effects of associating Quercus robur L. and Alnus cordata Loisel. On plantation productivity and water use efficiency. *Forest ecology and management*, *391*, 106–114.

Bernetti, G. (1995). *Selvicoltura speciale-UTET*. Torino.

Bielak, K., Dudzińska, M., & Pretzsch, H. (2014). Mixed stands of Scots pine (Pinus sylvestris L.) and Norway spruce [Picea abies (L.) Karst] can be more productive than monocultures. Evidence from over 100 years of observation of long-term experiments. *Forest Systems*, *23*(3), 573–589.

Binkley, D., Dunkin, K. A., DeBell, D., & Ryan, M. G. (1992). Production and nutrient cycling in mixed plantations of Eucalyptus and Albizia in Hawaii. *Forest Science*, *38*(2), 393–408.

Binkley, D., Stape, J. L., & Ryan, M. G. (2004). Thinking about efficiency of resource use in forests. *Forest Ecology and Management*, *193*(1–2), 5–16.

Biraghi, A. (1949). Il disseccamento degli abeti di Vallombrosa. *L'Italia forestale e montana*, *4*(3), 141–151.

Blomquist, M., Herrera, S. L., Hofmann, J., Beram, R. C., Cleary, M., & Rönnberg, J. (2020). Size matters but is big always better? Effectiveness of urea and Phlebiopsis gigantea as treatment against Heterobasidion on Picea abies stumps of variable size. *Forest Ecology and Management*, *462*, 117998.

Borghetti, M., & Giannini, R. (1984). Indagini sulla rinnovazione naturale nei boschi puri e misti di abete bianco dell'Appennino centro-meridionale. *L'Italia Forestale e Montana*, *39*(4), 161–184.

Bravo-Oviedo, A., Pretzsch, H., & del Río, M. (2018). *Dynamics, silviculture and management of mixed forests*. Springer.

Brūna, L., Lione, G., Kenigsvalde, K., Burņeviča, N., Zaļuma, A., Kļaviņa, D., Gaitnieks, T., & Gonthier, P. (2021). Inferences on the Susceptibility of Wood of Different Tree Species to Heterobasidion annosum Sensu Lato Primary Infections and on the Range of Pathogen Spores Dispersal. *Forests*, *12*(7), 854.

Bunn, A. G. (2010). Statistical and visual crossdating in R using the dplR library. *Dendrochronologia*, *28*(4), 251–258.

Calistri, I. (1962). Studio dell’influenza dell’andamento pluviometrico sull’ampiezza degli anelli legnosi in Abies alba Mill. *Italia Forestale e Montana*, *17*, 148–161.

Caminiti, F., Gugliotta, O., Mercurio, R., Modica, G., & Spampinato, G. (2002). Primo contributo per lo studio dei tipi forestali nel Parco Nazionale dell’Aspromonte. *Annali dell’Accademia Italiana di Scienze Forestali*, *51*, 159–218.

Cantiani, M. (1967). L’influenza dell’andamento stagionale sull’evoluzione dell’accrescimento della douglasia. *dell’abete bianco e di altre specie forestali. Annali dell’Accade-mia Italiana di Scienze Forestali*, *16*, 41–60.

Capretti, P. (1986). Nuovi reperti di Heterobasidion annosum (Fries.) Bref. *Monti e boschi*, *5*, 34–37.

Capretti, P. (1990). *Main diseases of Pinus sylvestris L. in Italy.*

Cherubini, P., Fontana, G., Rigling, D., Dobbertin, M., Brang, P., & Innes, J. L. (2002). Tree-life history prior to death: Two fungal root pathogens affect tree-ring growth differently. *Journal of ecology*, *90*(5), 839–850.

Chiarugi, A. (1936). Ricerche Sulla Vegetazione Dell’etruria Marittima: I.—Cicli Forestali Postglaciali Nell’appennino Etrusco Attraverso L’analist Pollinica Di Torbe E Depositi Lacustri Presso L’alpe Delle Tre Potenze E Il M. Rondinaio. *Plant Biosystem*, *43*(1), 3–61.

Chomel, M., DesRochers, A., Baldy, V., Larchevêque, M., & Gauquelin, T. (2014). Non-additive effects of mixing hybrid poplar and white spruce on aboveground and soil carbon storage in boreal plantations. *Forest ecology and management*, *328*, 292–299.

Ciancio, O., Iovino, F., Menguzzato, G., & Mirabella, A. (1985). *L’Abete (Abies alba Mill.) in Calabria: Possibilità e limiti di diffusione e ridiffusione*.

Ciancio, O., & Nocentini, S. (1996). Systemic silviculture: Scientific and technical consequences. La selvicoltura sistemica: Conseguenze scientifiche e tecniche. *L’Italia forestale e montana*, *51*(2), 112–130.

Coll, L., Ameztegui, A., Collet, C., Löf, M., Mason, B., Pach, M., Verheyen, K., Abrudan, I., Barbati, A., & Barreiro, S. (2018). Knowledge gaps about mixed forests: What do European forest managers want to know and what answers can science provide? *Forest Ecology and Management*, *407*, 106–115.

Condés, S., Del Rio, M., & Sterba, H. (2013). Mixing effect on volume growth of Fagus sylvatica and Pinus sylvestris is modulated by stand density. *Forest Ecology and Management*, *292*, 86–95.

Conte, E., Lombardi, F., Battipaglia, G., Palombo, C., Altieri, S., La Porta, N., Marchetti, M., & Tognetti, R. (2018). Growth dynamics, climate sensitivity and water use efficiency in pure vs. Mixed pine and beech stands in Trentino (Italy). *Forest Ecology and Management*, *409*, 707–718.

Cook, E., & Kairiukstis, L. (1990). Methods ofdendrochronology. *Applications in the Environmental*.

Creber, G. T., & Ash, S. R. (1990). Evidence of widespread fungal attack on Upper Triassic trees in the southwestern USA. *Review of Palaeobotany and Palynology*, *63*(3–4), 189–195.

Cudlín, P., Klopčič, M., Tognetti, R., Máli&, F., Alados, C. L., Bebi, P., Grunewald, K., Zhiyanski, M., Andonowski, V., & La Porta, N. (2017). Drivers of treeline shift in different European mountains. *Climate Research*, *73*(1–2), 135–150.

Dagnelie, P. (1973). *Théorie et méthodes statistiques. Applications agronomiques. 1, La statistique descriptive et les fondements de l'inférence statistique*. J. Duculot.

Dagnelie, P. (1975). Théorie et méthodes statistiques. Vol. 2. Les Presses Agronomiques, Gembloux, Belgium. *Théorie et méthodes statistiques. Vol. 2. Les Presses Agronomiques, Gembloux, Belgium.*

Dalman, K., Olson, Å., & Stenlid, J. (2010). Evolutionary history of the conifer root rot fungus Heterobasidion annosum sensu lato. *Molecular Ecology*, *19*(22), 4979–4993.

Del Favero, R. (1990). *Nuovi orientamenti nell'assestamento forestale*. Atti Corso di Aggiornamento Professionale Quadrifoglio Dolomiti, Belluno.

Delatour, C. (1972). Le Fomes annosus. *Revue forestière française*, *24*(1), 20–38.

Di Tommaso, P. L. (1976). [Effects of a heavy thinning on the underwood of an even-aged silver fir stand [Italy]].[Italian]. *Italia Forestale e Montana*.

Drapier, J. (1985). Les difficultés de régénération naturelle du Sapin (Abies alba Mill.) dans les Vosges. Etude écologique. *Revue forestière française, 37*(1), 45–55.

Dynesius, M., & Jonsson, B. G. (1991). Dating uprooted trees: Comparison and application of eight methods in a boreal forest. *Canadian Journal of Forest Research*, *21*(5), 655–665.

Edel, V., Steinberg, C., Gautheron, N., & Alabouvette, C. (2000). Ribosomal DNA-targeted oligonucleotide probe and PCR assay specific for Fusarium oxysporum. *Mycological Research*, *104*(5), 518–526.

Farjon, A. (2017). Abies alba. *The IUCN Red List of Threatened Species*.

Ferrarini, E. (1962). Analisi polliniche di depositi lacustri dell'Appennino lunigianese con osservazioni sulla vegetazione attuale. *Ann. Acc. Ital. Sc. Forest, 11*, 61–95.

Ferrarini, E., & Padula, M. (1969). Indagini sui pollini fossili di alcune località della Calabria (Sila Piccola e Serre) con osservazioni sulla vegetazione attuale. *Plant Biosystem*, *103*(6), 547–595.

Field, E., Petrokofsky, G., & Koricheva, J. (s.d.). *What are the impacts of mixed forest stands on forest pathogens? A systematic review protocol*.

Fornaini, L. (1804). *Della coltivazione degli abeti*. Dissertazione.

Fornaini, L. (1825). *Saggio sopra l'utilità di ben conservare e preservare le foreste*. Stamperia Gasparo Ricci, Firenze.

Forrester, D. I. (2014). The spatial and temporal dynamics of species interactions in mixed-species forests: From pattern to process. *Forest Ecology and Management*, *312*, 282–292.

Forrester, D. I., Benneter, A., Bouriaud, O., & Bauhus, J. (2017). Diversity and competition influence tree allometric relationships–Developing functions for mixed-species forests. *Journal of Ecology*, *105*(3), 761–774.

Forrester, D. I., Lancaster, K., Collopy, J. J., Warren, C. R., & Tausz, M. (2012). Photosynthetic capacity of Eucalyptus globulus is higher when grown in mixture with Acacia mearnsii. *Trees*, *26*(4), 1203–1213.

Fritts, H. C. (1976). Tree rings and climate Academic Press. *New York*.

Gabbrielli, A., Marca, O. la, & Paci, M. (1990). Silver fir in the Appennines. *Cellulosa e Carta*, *41*(6), 2–16.

Gaitnieks, T., Bruna, L., Zaluma, A., Burnevica, N., Klavina, D., Legzdina, L., Jansons, J., & Piri, T. (2021). Development of Heterobasidion spp. Fruit bodies on decayed Piecea abies. *Forest Ecology and Management*, *482*, 118835.

Gamfeldt, L., Snäll, T., Bagchi, R., Jonsson, M., Gustafsson, L., Kjellander, P., Ruiz-Jaen, M. C., Fröberg, M., Stendahl, J., & Philipson, C. D. (2013). Higher levels of multiple ecosystem services are found in forests with more tree species. *Nature communications*, *4*(1), 1–8.

Garbelotto, M., Lione, G., Martiniuc, A. V., & Gonthier, P. (2022). The alien invasive forest pathogen Heterobasidion irregulare is replacing the native Heterobasidion annosum. *Biological Invasions*, 1–15.

Gasparini, P., & Tabacchi, G. (2011). L'Inventario Nazionale delle Foreste e dei serbatoi forestali di Carbonio INFC 2005. *Secondo inventario forestale nazionale italiano. Metodi e risultati. Ministero delle Politiche Agricole, Alimentari e Forestali, 653*.

Gebauer, T., Horna, V., & Leuschner, C. (2012). Canopy transpiration of pure and mixed forest stands with variable abundance of European beech. *Journal of Hydrology*, *442*, 2–14.

Gentile, S. (1970). Sui faggeti dell'Italia meridionale. *Pavia Univ Lab Crittogam Atti*.

Gonthier, P., Anselmi, N., Capretti, P., Bussotti, F., Feducci, M., Giordano, L., Honorati, T., Lione, G. G., Luchi, N., & Michelozzi, M. (2014). An integrated approach to monitor and control the invasive fungal pathogen Heterobasidion irregulare in European forest stands. *11th Conference of the European Foundation for Plant Pathology, Healthy Plants–Healthy People*, 68–68.

Gonthier, P., Anselmi, N., Capretti, P., Bussotti, F., Feducci, M., Giordano, L., Honorati, T., Lione, G. G., Luchi, N., & Michelozzi, M. (2015). *Heterobasidion irregulare: Un patogeno forestale invasivo in Italia*.

Gonthier, P., Garbelotto, M., & Nicolotti, G. (2003). Swiss stone pine trees and spruce stumps represent an important habitat for Heterobasidion spp. In subalpine forests. *Forest Pathology*, *33*(3), 191–203.

Gonthier, P., Nicolotti, G., Linzer, R., Guglielmo, F., & Garbelotto, M. (2007). Invasion of European pine stands by a North American forest pathogen and its hybridization with a native interfertile taxon. *Molecular ecology*, *16*(7), 1389–1400.

Gori, Y., Cherubini, P., Camin, F., & La Porta, N. (2013). Fungal root pathogen (Heterobasidion parviporum) increases drought stress in Norway spruce stand at low elevation in the Alps. *European Journal of Forest Research*, *132*(4), 607–619.

Gradi, A. (1983). Declino e riespansione dell’abete bianco. *Economia Montana*, *15*(4), 16–23.

Grossiord, C., Granier, A., Gessler, A., Jucker, T., & Bonal, D. (2014). Does drought influence the relationship between biodiversity and ecosystem functioning in boreal forests? *Ecosystems*, *17*(3), 394–404.

Guntekin, E., Emiroglu, Z. G., & Yilmaz, T. (2013). Prediction of bending properties for turkish red Pine (Pinus brutia Ten.) lumber using stress wave method. *BioResources*, *8*(1), 231–237.

Gunulf, A., Rönnberg, J., & Berglund, M. (2012). Comparison of colonization capacity by asexual spores of Heterobasidion species in Norway spruce wood. *Forest Pathology*, *42*(4), 338–344.

Gunulf, A., Wang, L., Englund, J.-E., & Rönnberg, J. (2013). Secondary spread of Heterobasidion parviporum from small Norway spruce stumps to adjacent trees. *Forest Ecology and Management*, *287*, 1–8.

Gunulf Åberg, A., Witzell, J., & Rönnberg, J. (2016). Risk of False Positives during Sampling for Heterobasidion annosum sl. *Plant Disease*, *100*(1), 175–179.

Hammer, Ø., Harper, D. A. T., & Ryan, P. D. (2001). PAST-palaeontological statistics, ver. 1.89. *Palaeontol. electron*, *4*(1), 1–9.

Hanewinkel, M., Cullmann, D. A., Schelhaas, M.-J., Nabuurs, G.-J., & Zimmermann, N. E. (2013). Climate change may cause severe loss in the economic value of European forest land. *Nature climate change*, *3*(3), 203–207.

Hanlin, R. T. (1982). Conidiogenesis in Spiniger meineckellus. *Mycologia*, *74*(2), 236–241.

Hausser, K., & Troeger, R. (1967). Beitrag zur Frage der Massen und Wertleistung gepflanzter Weisstannen und Fichtenbestände auf gleichen Standorten. *Allgemeine Forst und Jagdzeitung*, *138*(7), 150–157.

Huber, B. (1943). Über die Sicherheit jahrringschronologischer Datierung. *Holz als Roh-und Werkstoff*, *6*(10), 263–268.

Huuskonen, S., Domisch, T., Finér, L., Hantula, J., Hynynen, J., Matala, J., Miina, J., Neuvonen, S., Nevalainen, S., & Niemistö, P. (2021). What is the potential for replacing monocultures with mixed-species stands to enhance ecosystem services in boreal forests in Fennoscandia? *Forest ecology and management*, *479*, 118558.

Ioos, R., Chrétien, P., Perrault, J., Jeandel, C., Dutech, C., Gonthier, P., Sillo, F., Hietala, A. M., Solheim, H., & Hubert, J. (2019). Multiplex real-time PCR assays for the detection and identification of Heterobasidion species attacking conifers in Europe. *Plant Pathology*, *68*(8), 1493–1507.

Iovino, F., Kramer, W., & Menguzzato, G. (1989). [Temperature and air and soil moisture of silver fir forests in the Serra San Bruno area (Calabria/Italy)].[German]. *Forstarchiv*.

Iovino, F., Menguzzato, G., & Veltri, A. (1988). *Studio delle condizioni termoigrometriche dell'aria e del suolo melle abetine di serra San Bruno*.

IPCC. (2017). Meeting report of the intergovernmental panel on climate change expert meeting on mitigation, sustainability and climate stabilization scenarios. In *IPCC Working Group III technical support unit*. Imperial College London London.

Jactel, H., Bauhus, J., Boberg, J., Bonal, D., Castagneyrol, B., Gardiner, B., Gonzalez-Olabarria, J. R., Koricheva, J., Meurisse, N., & Brockerhoff, E. G. (2017). Tree diversity drives forest stand resistance to natural disturbances. *Current Forestry Reports*, *3*(3), 223–243.

Kaennel, M., & Schweingruber, F. H. (1995). *Multilingual glossary of dendrochronology: Terms and definitions in english, german, french, spanish, italian, portugese and russian*. Paul Haupt.

Kanowski, J., Catterall, C. P., & Wardell-Johnson, G. W. (2005). Consequences of broadscale timber plantations for biodiversity in cleared rainforest landscapes of tropical and subtropical Australia. *Forest Ecology and Management*, *208*(1–3), 359–372.

Kelty, M. J. (1992). Comparative productivity of monocultures and mixed-species stands. In *The ecology and silviculture of mixed-species forests* (pp. 125–141). Springer.

Kelty, M. J. (2006). The role of species mixtures in plantation forestry. *Forest Ecology and Management*, *233*(2–3), 195–204.

Kennel, R. (1966). Soziale Stellung, Nachbarschaft und Zuwachs. *Forstwissenschaftliches Centralblatt*, *85*(7), 193–204.

Korhonen, K. (1978). *Intersterility groups of Heterobasidion annosum.* (Vol. 94, Fascicolo 6).

Korhonen, K., Capretti, P., Moriondo, F., & Mugnai, L. (1989). A new breeding group of Heterobasidion annosum found in Europe. *Proceedings of the 7th. International Conference on Root And Butt Rots, Vernon& Victoria, Canada 1998pp*, *20*, 26.

Kovalchuk, A., Mukrimin, M., Zeng, Z., Raffaello, T., Liu, M., Kasanen, R., Sun, H., & Asiegbu, F. O. (2018). Mycobiome analysis of asymptomatic and symptomatic Norway spruce trees naturally infected by the conifer pathogens Heterobasidion spp. *Environmental microbiology reports*, *10*(5), 532–541.

Kral, F. (1989). Le vicende del popolamento forestale sulle Alpi italiane. *Italia Forestale e Montana*, *44*(2), 107–131.

Kramer, H. (1988). Waldwachstumslehre (Forest growth and yield science)(In German) Paul Parey. *Hamburg, Berlin*, *374*.

Kramer, W. (1984). *Die Weisstanne (Abies alba Mill.) in Kalabrien: Beobachtungen und Eindrücke. 1*.

Krisans, O., Matisons, R., Rust, S., Burnevica, N., Bruna, L., Elferts, D., Kalvane, L., & Jansons, A. (2020). Presence of root rot reduces stability of Norway spruce (Picea abies): Results of static pulling tests in Latvia. *Forests*, *11*(4), 416.

La Marca, O. (1979). Indagini auxometriche e selvicolturali su abete bianco (Abies alba Mill.) attaccato da Heterobasidion annosum (Fr.) Bref. *Annali-Accademia italiana di scienze forestali*.

La Porta, N., Belloni, V., & Rotino, G. L. (1998). Regeneration of transgenic eggplants (Solanum melongena L.) for a cysteine proteinase inhibitor. Capsicum & Eggplant Newsletter, 17, 92-95.

Liang, J., Crowther, T. W., Picard, N., Wiser, S., Zhou, M., Alberti, G., Schulze, E.-D., McGuire, A. D., Bozzato, F., & Pretzsch, H. (2016). Positive biodiversity-productivity relationship predominant in global forests. *Science*, *354*(6309), aaf8957.

Lindén, M., & Vollbrecht, G. (2002). Sensitivity of Picea abies to butt rot in pure stands and in mixed stands with Pinus sylvestris in southern Sweden. *Silva Fennica*, *36*(4).

Lindqvist, H., Koponen, H., & Valkonen, J. P. (1998). Peronospora sparsa on cultivated Rubus arcticus and its detection by PCR based on ITS sequences. *Plant Disease*, *82*(12), 1304–1311.

Lingg, W. (1986). *Ökologie der inneralpinen Weißtannenvorkommen (Abies alba Mill.) im Wallis (CH): Mit 3 Tab. U. 3 Tab. In Tasche*.

Liu, C. L. C., Kuchma, O., & Krutovsky, K. V. (2018). Mixed-species versus monocultures in plantation forestry: Development, benefits, ecosystem services and perspectives for the future. *Global Ecology and Conservation*, *15*, e00419.

Machado, J. S., Louzada, J. L., Santos, A. J., Nunes, L., Anjos, O., Rodrigues, J., Simões, R. M., & Pereira, H. (2014). Variation of wood density and mechanical properties of blackwood (Acacia melanoxylon R. Br.). *Materials & Design (1980-2015)*, *56*, 975–980.

MAF, I. (1988). Inventario Forestale Nazionale 1985. *Sintesi metodologica e risultati, Trento*.

Magin, R. (1954). Ertragskundliche Untersuchungen in montanen Mischwäldern. *Forstwissenschaftliches Centralblatt*, *73*(3), 103–113.

Magini, E. (1967). Ricerche sui fattori della rinnovazione naturale dell'abete bianco sull'Appennino. *L'Italia Forestale e Montana*, *22*(6), 261–270.

Marziliano, P. A., Barreca, L., Menguzzato, G., Nicolaci, A., Pelle, L., & Scuderi, A. (2011). Struttura e incrementi in boschi puri e misti di abete e faggio sull'Appennino calabrese. *L'Italia Forestale e Montana, 66*(1), 55–70.

Marziliano P.A., Tognetti R., Mercuri M., Labate A., Lombardi F., 2022. Balancing stand productivity and wood quality in chestnut coppices using chronosequence approach and productivity model. European Jornal of Forest Research 141: (1059–1072).

Meffe, G. K., & Carroll, C. R. (1997). *Contributors. 1997. Principles of conservation biology*. Sinauer Associates, Sunderland, Massachusetts.

Mercurio, I., & Mercurio, R. (2008). Effetto del regime luminoso sulla rinnovazione naturale dell'abete bianco (Abies alba Mill.) nel bosco di Archiforo, Calabria. *Forest@-Journal of Silviculture and Forest Ecology*, *5*(3), 171.

Mercurio, R., & Spampinato, G. (2006). *I tipi forestali delle Serre calabresi*. Laruffa.

Mettin, C. (1985). Betriebswirtschaftliche und ökologische Zusammenhänge zwischen Standortskraft und Leistung in Fichtenreinbeständen und Fichten/Buchen-Mischbeständen. *AFZ-DerWald*, *40*, 803–810.

Mitscherlich, G. (1967). *Ertragkundlich-ökologische Untersuchungen im Rein- und Mischbestand*. na.

Moriondo, F. (1999). *Introduzione alla patologia forestale*. UTET.

Moriondo, F., Capretti, P., Liperoti, A., & Mugnai, L. (1988). Preliminary observations on intersterility groups of Heterobasidion annosum in Italy. *Phytopathologia Mediterranea*, *27*(3), 178–179.

Moriondo, F., & Covassi, F. (1981). Tannensterben in Italien. *Forstwissenschaftliches Centralblatt*, *100*(1), 168–170.

Mugnai, L., & Capretti, P. (1989). Intersterility groups of Heterobasidion annosum (Fr.) Bref.: Some morphological differences in the basidiocarps. *Micologia Italiana*, *3*, 87–94.

Müller, M. M., Hamberg, L., Kuuskeri, J., LaPorta, N., Pavlov, I., & Korhonen, K. (2015). Respiration rate determinations suggest H eterobasidion parviporum subpopulations have potential to adapt to global warming. *Forest Pathology*, *45*(6), 515–524.

Müller, M. M., Kaitera, J., & Henttonen, H. M. (2018). Butt rot incidence in the northernmost distribution area of Heterobasidion in Finland. *Forest Ecology and Management*, *425*, 154–163.

Negri, G. (1943). Residui di una abetina originaria al M. *Amiata. Il pigelleto di Pian Castagnaio." Studi Etruschi*, *17*, 389–418.

Nocentini, S. (2005). Conservazione della complessità e della diversità biologica dei sistemi forestali. *L'Italia Forestale e Montana*, *60*(4), 341–349.

Orsomando, E. (1974). *La distribuzione dell'Epipogium aphyllum nell'Appennino con due nuove stazioni nel Parco Nazionale d'Abruzzo e nei Monti della Laga*.

Patrone, G. (1952). Piano di assestamento dei boschi del Comune di Agnone. *Piano di assestamento dei boschi del Comune di Agnone.*, 70–97.

Pavari, A. (1914). Sulla limitazione naturale del taglio saltuario. *L'Alpe*, *1*(10), 334–337.

Pavari, A. (1916). *Studio preliminare sulla coltura di specie forestali esotiche in Italia: Parte generale* (Vol. 1). M. Ricci.

Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., Blondel, M., Prettenhofer, P., Weiss, R., & Dubourg, V. (2011). Scikit-learn: Machine learning in Python. *the Journal of machine Learning research*, *12*, 2825–2830.

Pellicciaro, M., Lione, G., Giordano, L., & Gonthier, P. (2021). Biocontrol potential of Pseudomonas protegens against Heterobasidion species attacking conifers in Europe. *Biological Control*, *157*, 104583.

Pellicciaro, M., Padoan, E., Lione, G., Celi, L., & Gonthier, P. (2022). Pyoluteorin Produced by the Biocontrol Agent Pseudomonas protegens Is Involved in the Inhibition of Heterobasidion Species Present in Europe. *Pathogens, 11*(4), 391.

Petit, B., & Montagnini, F. (2006). Growth in pure and mixed plantations of tree species used in reforesting rural areas of the humid region of Costa Rica, Central America. *Forest Ecology and Management*, *233*(2–3), 338–343.

Petráš, R., Mecko, J., Bošeľa, M., & Šebeň, V. (2016). Wood quality and value production in mixed fir-spruce-beech stands: Long-term research in the Western Carpathians. *Lesnícky časopis-Forestry Journal*, *62*, 98–104.

Piotto, D. (2008). A meta-analysis comparing tree growth in monocultures and mixed plantations. *Forest Ecology and management*, *255*(3–4), 781–786.

Pizzigallo, V. (1941). Le abetine di Serra S. *Bruno.–Rivista Forest. Ital*, *3*, 179–189.

Porta, N. L., Apostolov, K., & Korhonen, K. (1998). Intersterility groups of Heterobasidion annosum and their host specificity in Bulgaria. *European journal of forest pathology*, *28*(1), 1–9.

Pretzsch, H., Bielak, K., Block, J., Bruchwald, A., Dieler, J., Ehrhart, H.-P., Kohnle, U., Nagel, J., Spellmann, H., & Zasada, M. (2013). Productivity of mixed versus pure stands of oak (Quercus petraea (Matt.) Liebl. And Quercus robur L.) and European beech (Fagus sylvatica L.) along an ecological gradient. *European Journal of Forest Research*, *132*(2), 263–280.

Pretzsch, H., Bielak, K., Bruchwald, A., Dieler, J., Dudzinska, M., Ehrhart, H. P., Jensen, A. M., Johannsen, V. K., Kohnle, U., & Nagel, J. (2013). Mischung und Produktivität von Waldbeständen. Ergebnisse langfristiger ertragskundlicher Versuche. *Allgemeine Forst-und Jagdzeitung*, *184*(7/8), 177–196.

Pretzsch, H., Block, J., Dieler, J., Dong, P. H., Kohnle, U., Nagel, J., Spellmann, H., & Zingg, A. (2010). Comparison between the productivity of pure and mixed stands of Norway spruce and European beech along an ecological gradient. *Annals of Forest Science, 67*(7), 712.

Pretzsch, H., & Rais, A. (2016). Wood quality in complex forests versus even-aged monocultures: Review and perspectives. *Wood science and technology, 50*(4), 845–880.

Pretzsch, H., & Schütze, G. (2009). Transgressive overyielding in mixed compared with pure stands of Norway spruce and European beech in Central Europe: Evidence on stand level and explanation on individual tree level. *European Journal of Forest Research*, *128*(2), 183–204.

Pretzsch, H., & Schütze, G. (2016). Effect of tree species mixing on the size structure, density, and yield of forest stands. *European journal of forest research*, *135*(1), 1–22.

Proto, A. R., Macrì, G., Bernardini, V., Russo, D., & Zimbalatti, G. (2017). Acoustic evaluation of wood quality with a non-destructive method in standing trees: A first survey in Italy. *iForest-Biogeosciences and Forestry*, *10*(4), 700.

Pukkala, T. (2017). Effect of species composition on ecosystem services in European boreal forest. *J. For. Res.* 29, 261-272.

Quézel, P., Barbéro, M., Benabid, A., & Rivas-Martinez, S. (1995). Les structures de végétation arborées à Acacia sur le revers méridional de l’Anti-Atlas et dans la vallée inférieure du Draa (Maroc). *Phytocoenologia*, 279–304.

Richards, A. E., Forrester, D. I., Bauhus, J., & Scherer-Lorenzen, M. (2010). The influence of mixed tree plantations on the nutrition of individual species: A review. *Tree Physiology*, *30*(9), 1192–1208.

Rishbeth, J. (1951). Observations on the biology of fomes annosus, with particular reference to east Anglian pine plantations: II. Spore production, stump infection, and saprophytic activity in stumps. *Annals of botany*, *15*(1), 1–22.

Russo, D., Marziliano, P. A., Macrì, G., Zimbalatti, G., Tognetti, R., & Lombardi, F. (2019). Tree growth and wood quality in pure vs. Mixed-species stands of European beech and Calabrian pine in Mediterranean mountain forests. *Forests*, *11*(1), 6.

Saha, S., Kuehne, C., Kohnle, U., Brang, P., Ehring, A., Geisel, J., Leder, B., Muth, M., Petersen, R., & Peter, J. (2012). Growth and quality of young oaks (Quercus robur and Quercus petraea) grown in cluster plantings in central Europe: A weighted meta-analysis. *Forest Ecology and Management*, *283*, 106–118.

Saranpää, P. (2003). Wood density and growth. *Wood quality and its biological basis*, 87–117.

Shain, L. (1971a). Phenolic extractives in Norway spruce and their effects on Fomes annosus. *Phytopathology*, *61*, 841–845.

Shain, L. (1971b). The response of sapwood of Norway spruce to infection by Fomes annosus. *Phytopathology*, *61*(3), 301–307.

Sibilia, C. (1952). Conseguenze della persistente siccità dell’ultimo decennio sui nostri boschi. *L’Italia Forestale e Montana*, *7*(4), 161–167.

Spiecker, H. (1986). Growth of fir (Abies alba Mill.) and spruce (Picea abies Karst.) in selection forest research plots of the Black Forest from 1950 to 1984. *Allgemeine Forst-und Jagdzeitung*, *157*(8), 152–164.

Susmel, L. (1959). Ecologia, biologia e possibilità attuali di coltivazione dell'abete bianco (Abies alba Mill.) nell'Appennino Centro Meridionale. *Annali dell'Accademia Italiana di Scienze Forestali*, *8*, 165–202.

Taxonomy, S. (1999). A basic system of soil classification for making and interpreting soil surveys. *Agriculture handbook*, *436*, 96–105.

Teder, M., Pilt, K., Miljan, M., Lainurm, M., & Kruuda, R. (2011). Overview of some non-destructive methods for in-situ assessment of structural timber. *Proceedings of the 3rd International Conference Civil Engineering, Jelgava, Latvia*, 12–13.

Torquato, L. P., Auty, D., Hernández, R. E., Duchesne, I., Pothier, D., & Achim, A. (2014). Black spruce trees from fire-origin stands have higher wood mechanical properties than those from older, irregular stands. *Canadian Journal of Forest Research*, *44*(2), 118–127.

Vainio, E. J., & Hantula, J. (2016). Taxonomy, biogeography and importance of Heterobasidion viruses. *Virus research*, *219*, 2–10.

Van der Nest, M. A., Olson, Å., Karlsson, M., Lind, M., Dalman, K., Brandström-Durling, M., Elfstrand, M., Wingfield, B. D., & Stenlid, J. (2014). Gene expression associated with intersterility in Heterobasidion. *Fungal Genetics and Biology*, *73*, 104–119.

Van der Plas, F., Manning, P., Soliveres, S., Allan, E., Scherer-Lorenzen, M., Verheyen, K., Wirth, C., Zavala, M. A., Ampoorter, E., & Baeten, L.

(2016). Biotic homogenization can decrease landscape-scale forest multifunctionality. *Proceedings of the National Academy of Sciences, 113*(13), 3557–3562.

Van Rossum, G., & Drake, F. L. (2000). Python reference manual. IUniverse Indiana. *Python reference manual: iUniverse Indiana*.

Volpini, C. (1961). *Piano di assestamento forestale del demanio di Serra San Bruno per il decennio 1957-1966*. Roma.

Von Siepmann, R. (1988). Intersterilitätsgruppen und Klone von Heterobasidion annosum-Isolaten aus Koniferen-Wurzel-und-Stammfäulen. *European Journal of Forest Pathology, 18*(2), 93–97.

Wang, L., Zhang, J., Drobyshev, I., Cleary, M., & Rönnberg, J. (2014). Incidence and impact of root infection by Heterobasidion spp., and the justification for preventative silvicultural measures on Scots pine trees: A case study in southern Sweden. *Forest ecology and management*, *315,* 153–159.

Wang, X. (2011). Fundamentals of acoustic measurements on trees and logs and their implication to field application. *In: Proceedings of the 17th lnternational Symposium on Nondestructive Tesing and Evaluation of Wood, 2011; pp 25-33; 2011.*, 25–33.

Wang, X. (2013). Acoustic measurements on trees and logs: A review and analysis. *Wood Science and Technology*, *47*(5), 965–975.

Werner, A., & Łakomy, P. (2002). Intraspecific variation in Heterobasidion annosum for mortality rate on Pinus sylvestris and Picea abies seedlings grown in pure culture. *Mycologia*, *94*(5), 856–861.

Wessels, C. B., Malan, F. S., & Rypstra, T. (2011). A review of measurement methods used on standing trees for the prediction of some mechanical properties of timber. *European Journal of Forest Research*, *130*(6), 881–893.

Yachi, S., & Loreau, M. (2007). Does complementary resource use enhance ecosystem functioning? A model of light competition in plant communities. *Ecology Letters*, *10*(1), 54–62.

Yde-Andersen, A. (1968). Fomes annosus in conifer stands of first and second rotation. *Internat Conf Fomes Annosus Proc*.

Zeller, L., Ammer, C., Annighöfer, P., Biber, P., Marshall, J., Schütze, G., del Río Gaztelurrutia, M., & Pretzsch, H. (2017). Tree ring wood density of Scots pine and European beech lower in mixed-species stands compared with monocultures. *Forest Ecology and Management*, *400*, 363–374.

Zhang, Y., Chen, H. Y., & Reich, P. B. (2012). Forest productivity increases with evenness, species richness and trait variation: A global meta-analysis. *Journal of ecology*, *100*(3), 742–749.

Appendix 1

European Journal of Forest Research
https://doi.org/10.1007/s10342-022-01488-y

ORIGINAL PAPER

Balancing stand productivity and wood quality in chestnut coppices using chronosequence approach and productivity model

Pasquale A. Marziliano[1] · **Roberto Tognetti**[2] · **Michele Mercuri**[1] · **Antonino Labate**[1] · **Fabio Lombardi**[1]

Received: 6 May 2022 / Revised: 13 July 2022 / Accepted: 29 July 2022

Abstract

Trade-offs between high stand productivity and good wood quality exist for chestnut coppices and related wood-based products. The main objective of this study was to determine the most suitable duration (in years) of cutting cycles that maximizes stand productivity and preserve wood quality of chestnut coppices in a Mediterranean setting. To this aim, a stand-level growth model was developed to verify if wood quality of chestnut coppices at different stand ages varies when the rotation period is modified. Wood quality and stand productivity were analysed, using a chronosequence approach, in coppice stands in Southern Italy characterized by four cutting cycles (15, 25, 30, and 50 years). Results implied that the culmination of the mean annual increment occurs at 28 years, while the current annual increment culminates 10 years earlier. The MOEd values revealed a negative correlation with shoot age; however, a cutting cycle between 25 and 30 years might represent the best compromise for balancing stand productivity and wood quality. Results are discussed in the context of adaptive forest management.

Keywords Castanea sativa Mill. · MOED · Cutting cycless · Stand productivity · Modeling

Introduction

Forests in the Mediterranean Europe are mainly located in mountain and inner areas and provide a range of ecosystem services with important social–ecological functions (Scarascia-Mugnozza et al. 2000; Pastorella et al. 2016). Coppice forests represent a relevant part of these landscapes (Cutini et al. 2021). The coppice system is extremely efficient, since it offers the benefits of simplified and flexible silvicultural approaches, fast tree regeneration, prompt biomass production (and carbon sequestration), and short cutting cycles and high stress tolerance (Moscatelli et al. 2007). In the second half of the twentieth century, coppice products (firewood, charcoal, paling) have suffered from the competition of industrial products, which has resulted in a decreasing interest on this traditional management system (Hédl et al. 2010). However, growing interests for renewable energy sources and wood products currently make coppices a promising multipurpose silvicultural system for addressing risks associated with climate change and related disturbances (e.g., drought spells, forest fires) in agricultural and forest landscapes of Mediterranean mountains and inner areas, while contributing to community resilience and halting land degradation (Kelly et al. 2015).

Coppice forests have gained a renewed interest in many European countries (Rydberg 2000). The main reason for this are as follows: (1) the increasing importance of renewable energy sources as a substitute for fossil fuels, (2) the preservation of coppice forests as an historical landscape element often characterized by a high nature conservation value (Buckley 1992; Lanuv 2007; Scherzinger 1996; Parisi et al. 2020), (3) the high ecological value of these ecosystems, useful to support a wide diversity of fauna and flora (Zlatanov et al. 2013). These reasons (economic, social, and ecological) may open new perspectives for managing the abandoned coppice stands in many areas of Southern Europe and beyond.

In this context, chestnut (*Castanea sativa* Mill.), as one of the most important tree species of Southern Europe (Fabbio

---

Communicated by Miren del Río.

✉ Pasquale A. Marziliano
pasquale.marziliano@unirc.it

[1] Department of AGRARIA, Mediterranean University of Reggio Calabria, 89122 Reggio Calabria, Italy

[2] Department of Agricultural, Environmental and Food Sciences, University of Molise, 86100 Campobasso, Italy

2016), has the potential to deliver the multifunctional services of climate smart forestry in mountain areas (Bowditch et al. 2020). Chestnut forests cover more than 2.5 million hectares (Conedera et al. 2016), and their expansion through European centuries over time has been favoured by the multiple uses of chestnut-derived wood and non-wood forest products. Chestnut forests are usually managed as coppice stands, with or without reserve trees, but also as traditional fruit orchards, often in the framework of agroforestry systems. In Italy, chestnut agroforestry systems cover an area of about 800,000 ha (Giannini et al. 2014), 70% of which are managed as coppices, while the remaining 30% are managed as orchards for fruit production (Greco et al. 2018), supporting agricultural systems and rural communities in mountain areas.

Traditionally, in the Mediterranean Europe, chestnut has been successfully managed as coppice forest, which has led to the development of sustainable silvicultural modules, reflecting the capacity of the coppice system to adapt to the market needs and environmental challenges of mountain areas (Ciancio et al. 2004; Patricio et al. 2005). Indeed, the flexibility and reversibility of this silvicultural system meet the market requirements, without compromising the resprouting ability and the provisioning of services (Manetti et al. 2006), e.g., slope stabilization. Depending on the social–ecological conditions, chestnut coppices can be either managed with short rotation periods (12–15–20 years) or with medium-to-long cutting cycles (25–30–50 years) to process a variety of wood-based products, including wood for pole, timber for sawing, firewood, tool handles, and fencing material (Manetti et al. 2009).

Recently, several studies have focused on the effect of coppicing on the overall productivity (and, therefore, carbon sequestration and stock capacity) of chestnut forests (Kneifl et al. 2015; Manetti et al. 2016; Esteban et al. 2018; Marcolin et al. 2020). However, in Europe, there has been less emphasis on investigating growth and yield models in chestnut coppices and wood quality of coppiced trees, with few exceptions based on non-destructive technologies (NDTs) (e.g., Russo et al. 2019, 2020). Referring to the first point, in chestnut coppices in northwest Spain, Menéndez-Miguélez et al. (2014) have developed an interesting and useful model system made by a taper function, a total volume equation, and a merchantable volume equation. On the other hand, the ecological behaviour and productive capacity of chestnut coppices are key factors in predicting tree growth and selecting management approach, especially in areas where timber production is the primary objective (Menéndez-Miguélez et al. (2015). With reference to the second point, stress wave-based non-destructive acoustic techniques have resulted in very useful methods for predicting the mechanical properties of woody materials (Guntekin et al. 2013). Among the parameters measurable by acoustic methods, the most important are the modulus of elasticity of wood (MOE) and the dynamic modulus of elasticity (MOEd), being related to wood anatomy and tree physiology. These parameters are fundamental for the evaluation of wood quality, providing information on the resistance to deflection and the stiffness of material (Teder et al. 2011; Wessels et al. 2011).

To answer questions on the effect of different management approaches and environmental factors on wood quality for a specific forest type in homogeneous environmental conditions, the history of tree growth can be reconstructed along a chronosequence. Chronosequences, assuming space-for-time substitution, aim to infer temporal dynamics from measurements at sites of different ages but similar in land-use histories (Aide et al. 2000; Hedde et al. 2008; Pawson et al. 2009). In forestry, a chronosequence is a set of forest stands that share similar attributes but are of different ages (Johnson and Miyanishi 2008), reflecting a time sequence (Salisbury 1952; Pickett 1989). The key assumption of chronosequences is that each of the sites represents different developmental stages, with the same initial conditions. When there are demonstrable linkages between stages (i.e., the successional trajectory is predictable), chronosequences provide a useful approach to study temporal changes in a long-term perspective (Walker et al. 2010).

In this study, a stand-level growth and yield model for chestnut coppices was developed using a chronosequence approach. Chestnut coppices of different ages, growing in homogeneous environmental (biotic and abiotic) conditions, were selected in the “Aspromonte” National Park (Calabria, Southern Italy). The model, replicable in other geographical contexts, was implemented to verify if the quality and quantity of wood-based products vary with changing rotation periods. More specifically, variation in wood quality and stand productivity of coppiced chestnut was analysed for four different cutting cycles, referring to 15, 25, 30, and 50 years (hereafter C15, C25, C30, and C50, respectively). These four cutting cycles were considered because they are the most used in the chestnut coppices of Calabria. Our study was aimed to answer the following main question: Does the extension of rotation periods induce a variation of wood quality and tree growth of coppiced chestnut? We hypothesized that wood production increased along with the elongation of rotation timespan in chestnut coppice stands in a typical Mediterranean mountainous region, without impairing wood quality.

Materials and methods

Study area

The study area is located in Southern Italy (Calabria), within of the “Aspromonte” National Park, (570,304.25 E; 4,226,245.48 N) at an altitude ranging between 800 and 1100 m a.s.l. (Fig. 1). Soils developed from igneous and metamorphic rocks and are classified as Umbrisols, Cambisols, and Leptosols (FAO 2014), with an udic soil regime moisture. The average annual rainfall is 1605 mm, and the mean annual temperature is 10.6 °C.

The studied forest stands were characterized by monospecific coppices dominated by chestnut, with an age varying from 6 to 50 years. Stands over 30 years of age were subjected to at least one thinning carried out between the 25th and 30th year of age. Altogether, the analysed plots were considered as chronosequences, based on the assumption that all coppice stands share similar biotic and abiotic conditions and disturbance legacies. These stands can be considered representative of most chestnut coppices, as widespread forest systems in the Mediterranean context.

Data collection and analysis

Data collection was realized in 44 plots located in 16 stands with tree age ranging from 6 to 50 years, arranged through a systematic sampling design. Table 1 shows the number of plots and their extent per each age class, with an extension ranging between 530 and 1200 m^2. All plots were chestnut coppices, placed along with chronosequences, following a systematic sample grid, with a total area of 120 ha. On average, each chronosequence was about 7 ha.

In each plot, the following parameters were recorded: (1) number of stools, (2) number of shoots per stump, (3) the diameter at breast height (DBH) of all the shoots, and (4) the total height of 20% of the shoots, homogeneously distributed across the different DBH classes. Table 2 shows the descriptive statistics (mean and standard deviation) of the variables measured in the chestnut coppice stands aged 15, 20, 30, and 50 years.

Table 1 Number of plots and plot area per each age class

Age class (years)	Number of plots	Plot area (m^2)
6	3	531
9	3	531
12	3	531
15	7	531
18	3	908
21	3	908
24	4	908
27	2	908
30	5	1257
33	2	1257
36	1	1257
39	1	1257
42	2	1257
45	1	1257
48	2	1257
50	2	1257

Furthermore, the MOEd was measured on each shoot with a height greater than 1.5 m in 7 plots for the age class of 15 years, in 4 plots for the age class of 25 years, in 5

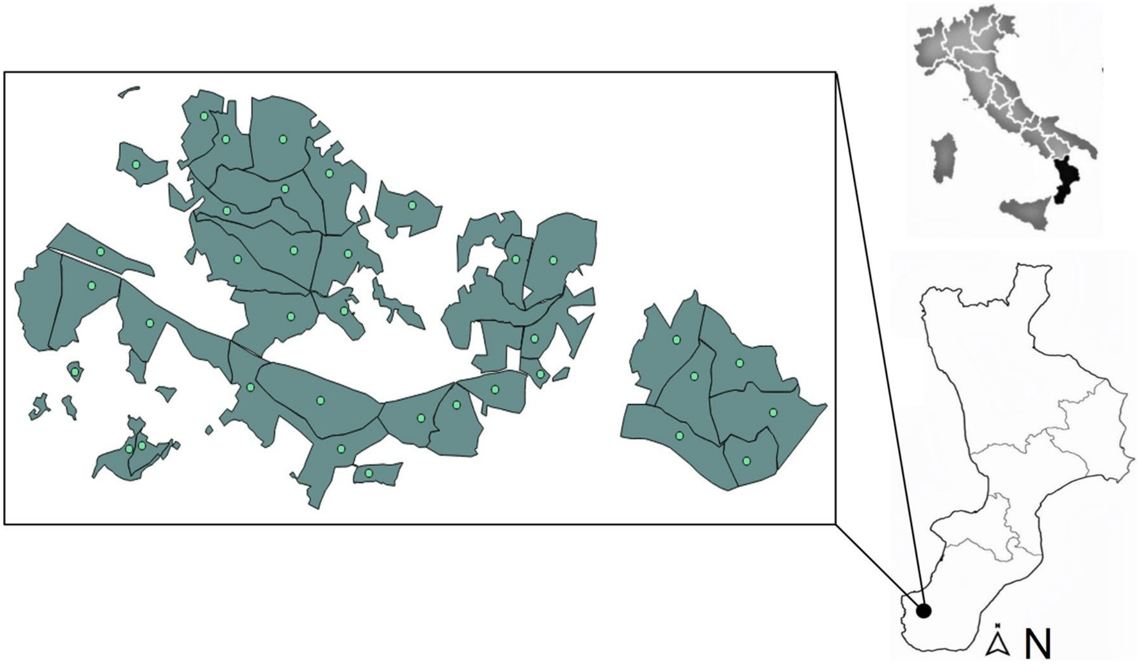


Fig. 1 Location of the study area in Southern Italy (Calabria) and the applied experimental design

plots for the age class of 30 years, and in 5 plots for the age class of 50 years, for a total of 1952 shoots.

To measure the acoustic velocity, the TreeSonic™ (Fakopp Enterprise, Agfalva, Hungary) was used inserting two sensor probes (a transmitting probe and a receiving probe) into the sapwood, then introducing the acoustic energy into the tree through a hammer impact (for further details, Vanninen et al. 1996; Divos 2010; Russo et al. 2019). Three measurements were carried out for each selected shoot and the average of the three recordings was used as the final transit time. The acoustic velocity was then calculated considering the distance between the two sensor probes and the time-of-flight (TOF) data using the following equation:

$$\mathrm{CT} = S/\mathrm{TOF} \tag{1}$$

where CT = tree acoustic velocity (m/s), S = distance between the two probes (sensors) (m), TOF = time-of-flight (s).

Afterwards, the MOEd was calculated, according to the following equation (Eq. 2):

$$\mathrm{MOEd} = \mathrm{WD}ij * \mathrm{CT2} \tag{2}$$

where WDij = wood density (kg m^{-3}), shared by DBH class (i) and age (j), and CT = velocity (m s^{-1}).

Wood density was determined on a subsample of shoots in the 15-, 25-, 30-, and 50-year-old stands; more in detail, we measured wood density on approximately 20% (390) of the total shoots (1952), considering all the diameter classes. In detail, woody cores were extracted at breast height (1.30 m) with a Pressler borer from stems, referring to each of the DBH classes at different ages. The fresh weight and wood volume were measured in the laboratory. The wood volume was calculated with the cylinder formula, measuring the diameter of woody cores with a small electronic precision calliper, and their length with a centimetre to the nearest millimetre. Samples were then weighed to the nearest 0.01 g with an electronic balance. Oven drying of all samples was done at 105 °C to constant weight. Wood density (kg m^{-3}) was calculated by dividing the dry mass by the sample volume.

Statistical analysis

Stand-level growth and yield models were fitted based on (1) the average number of shoots per ha, (2) the diameter-height-age relationship, and (3) the average values of basal area. All models were fitted by the ordinary least square's method using '*lm*' function for the R programming language (R Core Team 2019). The criteria used for comparing the models were based on the analysis of goodness-of-fit statistics. The adjusted coefficient of determination (R^2 adj), root mean square error (RMSE), and Akaike's information criterion were used to select the best candidate models. In all models, the absence of multicollinearity among the predictors was tested by posing a variance inflation factor (VIF = 3) (Zuur et al. 2010).

In detail, starting from the number of shoots per ha (NS) recorded in each plot and considering Age as independent variable, the best model obtained through a stepwise procedure was the following equation:

$$\mathrm{NS} = \beta_0 \cdot \mathrm{AGE}^{\beta_1} \tag{3}$$

The equation coefficients were determined analytically after a logarithmic linearization:

$$\mathrm{Ln(NS)} = \beta_0 + \beta_1 \cdot \ln(\mathrm{AGE}) \tag{4}$$

where NS is the number of shoots per ha and AGE represents the stand age (years).

The diameter-height-age model, according to the approach used by Clutter et al. (1983) and Marziliano et al. (2013), was derived using the following function (Eq. (4)):

$$\ln(\mathrm{Ht}) = \beta_2 + \beta_3 \cdot NS + \beta_4 \cdot \frac{1}{\mathrm{Age}} + \beta_5 \cdot \frac{1}{dbh} + \beta_6 \cdot \frac{1}{dbh \cdot \mathrm{Age}} \tag{5}$$

where Ht = total shoot height (m), NS = number of shoots per ha, dbh = diameter at breast height (cm), AGE = stand age (years).

To estimate the development in basal area, we used the average tree basal area (g) as dependent variable, since the NS (density expression) is structurally included in the stand basal area (G); therefore, in this model, the independent variables were Age and NS per ha. Considering several combinations of these two variables, tested through

Table 2 Descriptive statistics (mean and standard deviation) of the variables measured in field (NS, number of shoots; DBH, diameter at breast height; Height, tree height; BA, basal area)

Age	NS (n ha^{-1})		DBH (cm)		Height (m)		BA (m^2 ha^{-1})	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
15	4164	329	8.8	4.1	10.7	2.3	25.16	2.20
25	2388	201	13.1	7.3	17.2	3.5	33.92	1.12
30	1959	120	16.9	4.8	19.8	3.5	41.31	4.63
50	1124	113	24.8	11.9	21.6	6.1	53.46	10.85

Table 3 Minimum size of assortments from chestnut coppices

Assortments	Diameter (cm)	Length (m)
Small assortments	2–15	1–2
Small poles	8–15	2–4
Big poles	10–18	2–5.5
Beams and boards	15–20	6–10

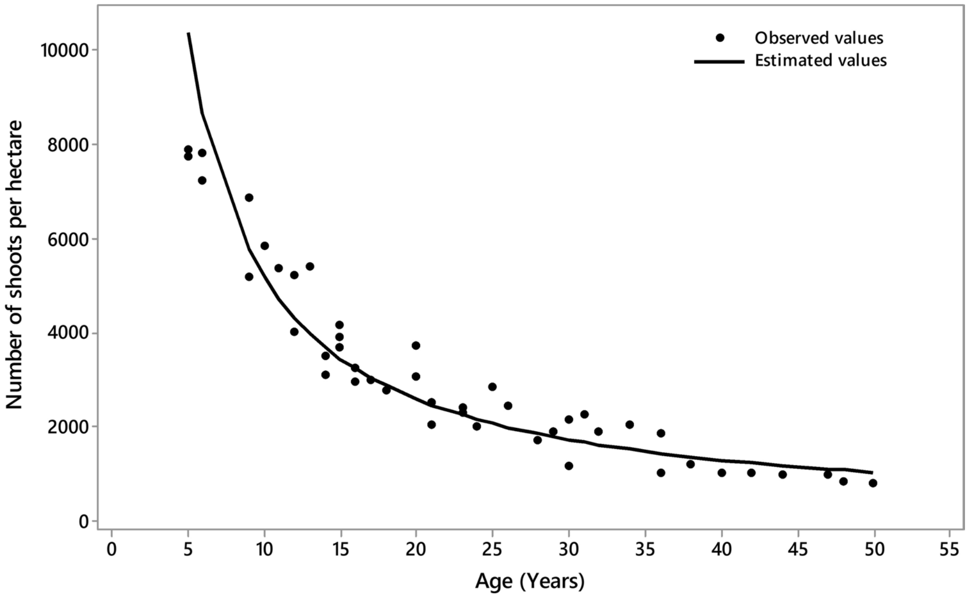


Fig. 2 Number of shoots per hectare, observed and estimated, in relation to the stand age

stepwise procedures, the best model resulted the following equation:

$$\ln(g) = \beta_7 + \beta_8 \cdot \frac{1}{\mathrm{Age}^2} + \beta_9 \cdot \mathrm{NS} \tag{6}$$

where g = average tree basal area (m^2), Age = Age (years), NS = number of shoots per ha.

The stand basal area (G) per ha was obtained multiplying the average basal area (g) estimated with Eq. (6), by the number of shoots (NS), occurring at each specific age, estimated with Eq. (4).

In the simulation of growth and yield of the coppice forests, once identified the initial conditions of the stand (number of shoots per hectare, age, stand mean diameter and height, basal area per hectare), stand volume and aboveground biomass were estimated using the equations reported for chestnut coppices in the National Forest Inventory protocols (INFC 2005; Tabacchi et al. 2011). More in detail, for each age class, the tree volume and aboveground biomass of the average tree (considering diameter and height of the shoots) were first estimated and then multiplied by the number of living shoots. Finally, the mean annual increment (MAI), the current annual increment (CAI), and the percentage of the current annual increment (PCAI) were calculated based on the estimates of tree volumes at the different ages.

Moreover, to estimate the assortments obtainable at different ages, a taper function developed for the chestnut coppices occurring in the region was used (Ciancio et al. 2004). For each diameter class i, using the taper function, we estimated the diameters along the stems at any height from the forest floor to the tree top. For each age, the assortments obtained for the diameter class i were multiplied by the number of trees belonging to the diameter class i. The assortments considered in this study were grouped in four categories (Table 3).

The analysis of variance (ANOVA) was carried out to test the differences in MOEd values obtained for the different ages and in relation to the DBH classes. The significance level of the differences was tested using Tukey's test. When the significance level (p value) was ≤ 0.05, the null hypothesis was rejected and significant differences in the means were accepted.

Results

Growth and yield model

Values of NS in relation to the stand age are reported in Fig. 2. Table 4 shows the regression statistics, in which all the significant parameters are detailed. Values of NS decreased as tree age increased. More in detail, NS values were very high in the first years after coppicing, then significantly decreased due to competition between shoots occurring on the same stumps. On average, from the occurrence of about 10,000 shoots per ha in the first years after coppicing (5 years), a significant reduction in NS values was observed, halving ten years after cutting. This trend was also confirmed in the following years, reaching about 2000 and 1000 shoots per ha at 25 and 50 years after cutting, respectively. Shoot mortality was very intense up to 20–25 years, significantly reducing afterwards (Fig. 2).

In Fig. 3, the tree height–diameter curve (i.e., the hypsometric relationship) is reported, showing the impacts of age factor. The regression coefficients of Eq. (5) and their standard errors show good accuracy of fitted curves. As expected, different tree heights were observed as ages varied: the height–diameter curve for young shoots (5 years old) was steeper than the other curves (e.g., from 20 to 50 years old).

Table 5 shows results of the growth and yield model obtained for stand age ranging from 5 to 50 years. In Fig. 4, the trend of stand volume over time is reported, as well as MAI and CAI. Tree volumes ranged from about 160 to 535 m^3 ha^{-1} for C15 and C50, respectively. The culmination of MAI occurred at 26 years (14 m^3 ha^{-1} $year^{-1}$), while CAI culminated about 10 years earlier (22 m^3 ha^{-1} $year^{-1}$). The percentage increment ranged from 26% at 6 years to 0.4% at 50 years.

Once the productivity and yield values were obtained, for each rotation cycle examined (C15, C25, C30 and C50), the obtainable wood assortments were estimated, through the taper function developed for the chestnut coppices in the Calabria Region (Ciancio et al. 2004). For C15 (the rotation period usually adopted), about 57% of the wood production consisted of small assortments (diameter ≤ 5 cm), while the small and large poles represented, respectively, 16 and 26% of the obtainable wood products. The yield of larger assortments was negligible (Fig. 4).

For C25 and particularly C30, a significant increase in large poles (respectively, of 42 and 54%) and a significant decrease in small assortments (respectively, of 31 and 28%) were observed. In both C25 and C30, higher quality assortments (beams and boards) were also observed, although to a lesser extent (about 8%). However, for C25 and C30, the prevailing assortment referred to small and large poles, equal to 61 and 63%, respectively (Fig. 4).

For C50, the percentage of beams and boards increased (of about 34%) representing, together with the large poles (35%), most of the obtainable assortments (Fig. 5).

Wood quality

Results obtained for the MOEd along the chronosequences are shown in Fig. 6. The ANOVA highlighted a significant effect of the length of rotation periods on MOEd values ($F_{3;1929} = 11.769$; $p \leq 0.001$). The highest values were obtained for C25 (on average 10,422 MPa), which significantly differed from C15 (on average 9536 MPa) and C50 (on average 8274 MPa). For cutting cycles ranging between 25 and 50 years, MOEd values decreased as the rotation period increased (Fig. 6).

Figure 7 reports MOEd values recorded for all samples across the different DBH classes. The ANOVA revealed that MOEd values decreased as the DBH increased ($F_{19;1929} = 1.995$; $p = 0.006$); more specifically, with DBH over 30–33 cm, significantly lower MOEd values were observed in comparison with smaller DBH classes.

In Fig. 8, MOEd variation across the different DBH classes and in relation to the stand age is shown. Generally, lower values of MOEd always occurred for larger DBH, while higher values characterized smaller DBH. The ANOVA showed a significant effect of the DBH classes on

Table 4 Estimation of each parameter used for Eqs. 3, 4, and 5

Equation		Parameters	SE parameters	R^2	RMSE
$\mathrm{Ln(NS)} = \beta_0 + \beta_1 \bullet \ln(\mathrm{AGE})$	β_0	10.9884	0.1384	0.926	0.187
	β_1	−1.0601	0.0456		
$\mathrm{Ln(Ht)} = \beta_2 + \beta_3 \bullet NS + \beta_4 \bullet \frac{1}{\mathrm{Age}}$	β_2	3.5479	0.0295	0.960	0.184
$+\beta_5 \bullet \frac{1}{dbh} + \beta_6 \bullet \frac{1}{dbh \bullet \mathrm{Age}}$	β_3	0.0002	<0.00001		
	β_4	−22.7110	0.9744		
	β_5	−6.1284	0.3351		
	β_6	10.2808	0.5148		
$\mathrm{Ln}(g) = \beta_7 + \beta_8 \bullet \frac{1}{\mathrm{Age}^2} + \beta_9 \bullet \mathrm{NS}$	β_7	−2.2041	0.3557	0.916	0.415
	β_8	125.186	58.825		
	β_9	−0.0009	0.00001		

Statistics for each equation are also reported. NS (number of shoots), HT (shoot height), g (average basal area). The equations for estimating the volume (dm^3) and aboveground biomass (kg) are those reported for chestnut coppice in the National Forest Inventory protocols (INFC 2005; Tabacchi et al. 2011)

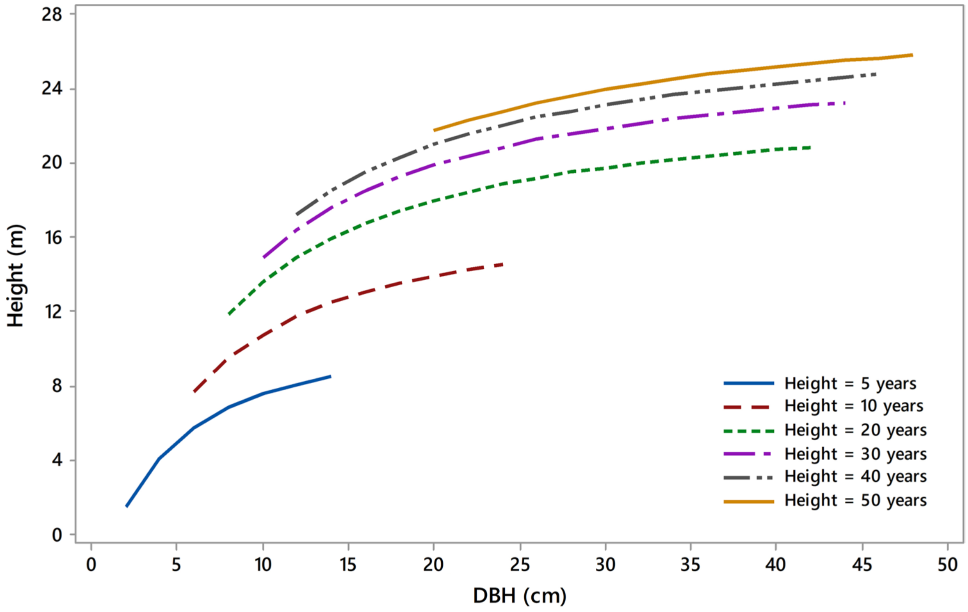


Fig. 3 Tree height–diameter relationship at different ages, estimated through Eq. 5

Table 5 Main structural characteristics estimated for the investigated stands at different ages (NS, number of shoots; Dg, average diameter of average basal area; Hg, tree average height; BA, basal area; MAI: Mean Annual Increment; CAI: Current Annual Increment; AGB: Above ground biomass)

Age	NS (n ha^{-1})	Dg (cm)	Hg (m)	BA (m^2 ha^{-1})	Vol (m^3 ha $^{-1}$)	MAI (m^3 ha^{-1} year^{-1})	CAI (m^3 ha^{-1} year^{-1})	AGB (Mg ha^{-1})
6	7720	4.7	1.6	13.16	21.68	3.61		24.28
8	5871	6.7	3.2	20.46	47.59	5.95	12.95	43.49
10	4748	8.6	5.1	27.79	88.92	8.89	20.67	67.70
12	3992	10.5	7.0	34.29	139.52	11.63	25.30	98.05
14	3448	12.1	8.8	39.72	193.76	13.84	27.12	128.24
16	3036	13.6	10.3	44.07	247.47	15.47	26.86	157.48
18	2715	14.9	11.7	47.46	298.10	16.56	25.31	184.56
20	2456	16.1	12.9	50.05	344.30	17.22	23.10	209.11
22	2243	17.2	13.9	51.97	385.56	17.53	20.63	230.32
24	2065	18.1	14.9	53.35	421.81	17.58	18.13	247.90
26	1913	19.0	15.7	54.30	453.29	17.43	15.74	263.58
28	1783	19.8	16.5	54.90	480.37	17.16	13.54	277.67
30	1670	20.5	17.2	55.22	503.45	16.78	11.54	288.40
32	1570	21.2	17.8	55.32	522.98	16.34	9.76	298.18
34	1482	21.8	18.3	55.24	539.35	15.86	8.18	304.47
36	1404	22.3	18.8	55.03	552.94	15.36	6.80	308.76
38	1333	22.9	19.3	54.70	564.11	14.84	5.58	315.98
40	1270	23.3	19.7	54.29	573.15	14.33	4.52	317.17
42	1212	23.8	20.1	53.81	580.34	13.82	3.60	321.21
44	1160	24.2	20.5	53.28	585.92	13.32	2.79	323.31
46	1112	24.6	20.8	52.71	590.10	12.83	2.09	324.23
48	1068	24.9	21.1	52.11	593.07	12.36	1.48	324.48
50	1027	25.3	21.4	51.49	594.97	11.90	0.95	324.60

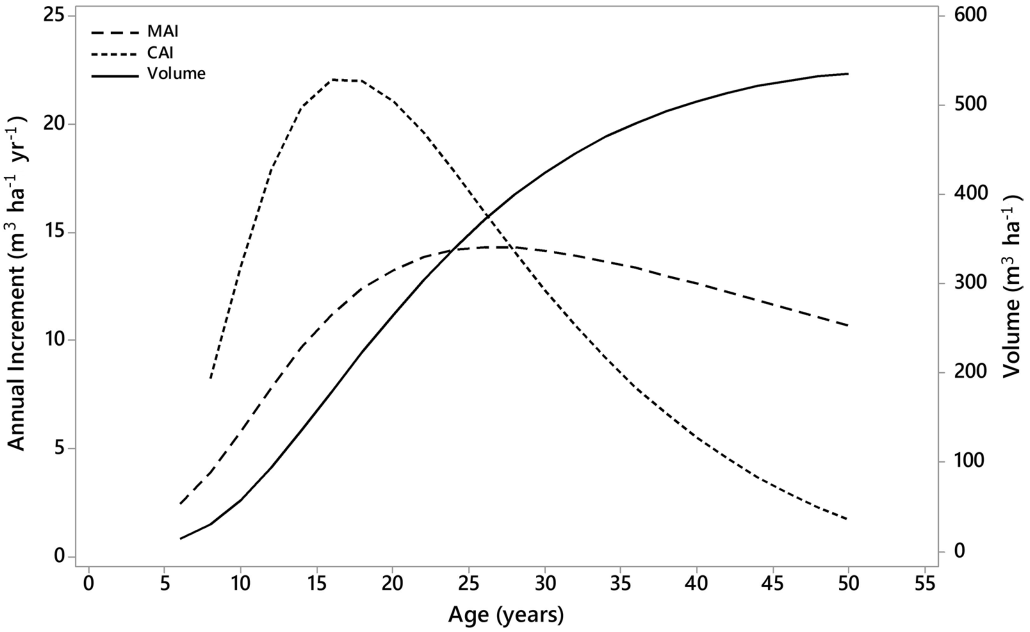


Fig. 4 Trends of the mean volumes, CAI, and MAI with increasing stand ages

MOEd values for C15 and C50, while minor effects were observed for C25 and C30.

The MOEd values decreased as the DBH increased for C15, with significant differences observed between the smallest and largest DBH. Particularly, for DBH higher than 12 cm, faster growth trends induced a significant decrease in MOEd values.

On the contrary, for C25 and C30, the MOEd values did not vary significantly when the DBH increased (Fig. 8). Results demonstrated that in C25, but also in C30, though to a lesser extent, the MOEd values aligned, making the wood quality uniform across the different DBH classes.

Finally, in C50, the MOEd values were not only significantly lower than in C25 and C30, but decreased significantly as the DBH increased, especially starting from the 30–33 cm DBH class.

Discussion

We developed and tested a stand-level growth and yield model for simulating the temporal development of the main structural traits for chestnut coppices in the Mediterranean climate change hotspot. Although this study was focused on chestnut coppices at the southernmost distribution limit of the species, predictions revealed high yield potential of these forest stands, confirmed by the considerable wood volumes and growth trends (MAI and CAI). Aspects of coppice productivity have been often ignored in common growth modelling approaches (Vanclay 1994; Pretzsch 2009; Weiskittel et al. 2011). Therefore, this empirical growth and yield model might provide useful insights in production modelling and forest planning applied to chestnut coppices and aimed at balancing stand productivity and wood quality. Nevertheless, the specific geographical context and the complexity of environmental setting in which data were collected may limit comparisons with other modelling exercises (Bernetti 1980; von Gadow and Hui 1999).

Angelini et al. (2013) estimated, on average, MAI ranging from about 7.2 to 13.0 m^3 ha^{-1} year^{-1} for 18–22-year-old chestnut coppices in Central Italy. Ciancio et al. (2004), for 15–45-year-old chestnut coppices in Calabria, found MAI ranging from 12 to 16 m^3 ha^{-1} year^{-1}. In Tuscany, Cutini (2001) recorded MAI of 17.6 and 12.8 m^3 ha^{-1} year^{-1}, for 15- and 38-year-old chestnut coppices, respectively. In an interesting study in chestnut coppices in North-Western Spain, Menéndez-Miguélez et al. (2016) showed, for high density coppices (7183 stems per hectare at 10 years and 880 stems per hectare at 60 years), MAI values from 8.1 m^3 ha^{-1} at the age of 35 for low site index to MAI values of 38.8 m^3 ha^{-1} at the age of 25 for high site index. Instead, for low density coppices (3366 stems per hectare at 10 years and 484 stems per hectare at 60 years), the MAI values ranged from 5.2 m^3 ha^{-1} at the age of 35–24.3 m^3 ha^{-1} at the age of 25. In both situations (low and high density), the culmination of MAI occurred at a younger age as the site index increased. It is worth noting that our sampling coppice stands, though differing in age, have similar disturbance legacies and occur on similar soil types and environmental conditions within the same climatic zone. Therefore, the commonly used site

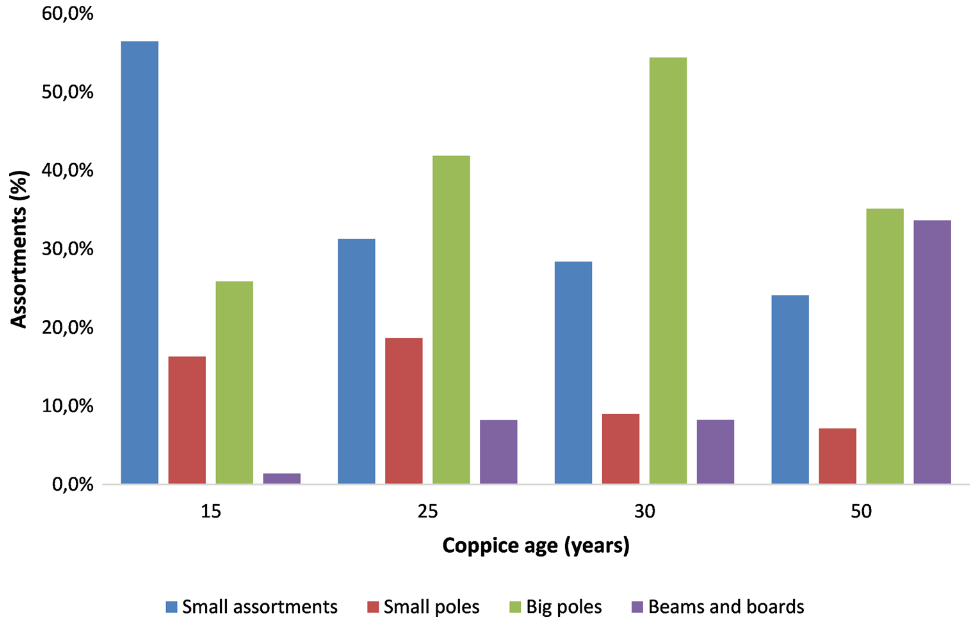


Fig. 5 Assortments for the four rotation periods (in percentage)

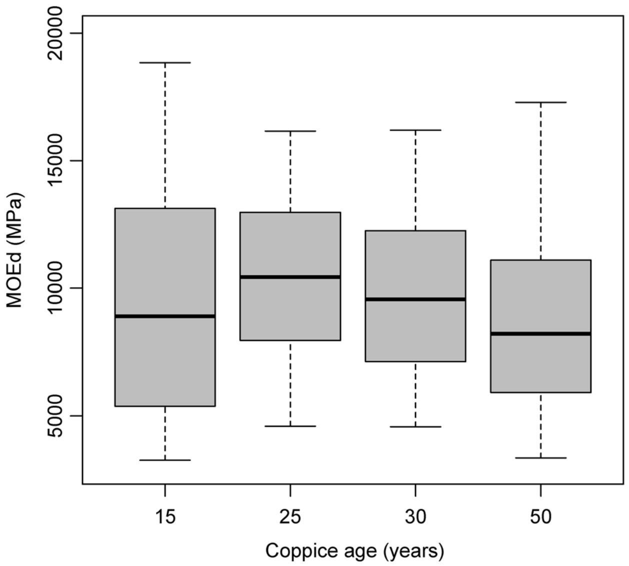


Fig. 6 Variation of the MOEd values at different stand age. The horizontal line indicates the median values; in the box, the lower limit corresponds to the value of the first quartile (Q1) of the distribution and the upper limit to the third quartile (Q3); the vertical lines (whiskers) delimit the intervals in which the lower values of Q1 (in the lower part) and the greater values of Q2 (in the upper part) are positioned

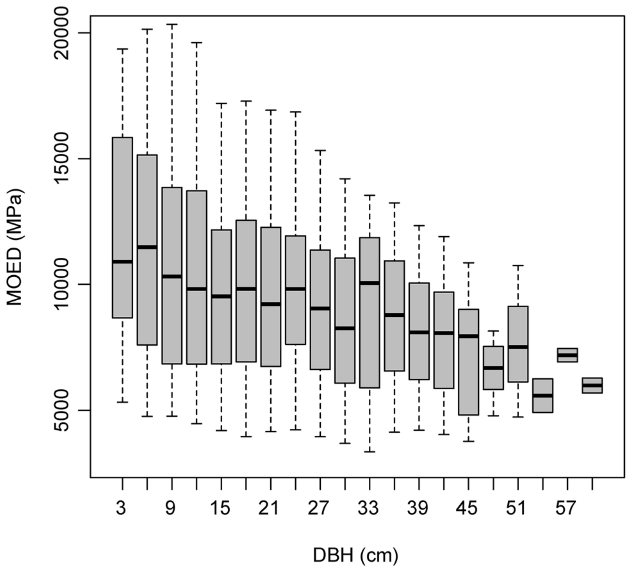


Fig. 7 MOEd values at different DBH, regardless of age. The horizontal line indicates the median values; in the box, the lower limit corresponds to the value of the first quartile (Q1) of the distribution and the upper limit to the third quartile (Q3); the vertical lines (whiskers) delimit the intervals in which the lower values of Q1 (in the lower part) and the greater values of Q2 (in the upper part) are positioned

index (estimated based on stand height) was not considered suitable to characterize site productivity for these homogeneous forest stands (Skovsgaard and Vanclay 2013).

In our study, the optimal rotation length that produces the maximum sustainable yield was to 25 years (about 18 m^3 ha^{-1} year^{-1}). The same rotation length was considered optimal in chestnut coppices in North-Western Spain (Menéndez-Miguélez et al. 2016). This rotation length (25 years) is lower than that reported by Cabrera and Ochoa (1997) (31 years) and by Elorrieta (1949) (30 years) in Spain, and even than those proposed by Bourgeois et al. (2004) and Lemaire (2008) for high quality timber in France (40–45 years). In addition, the MAI estimated in our study

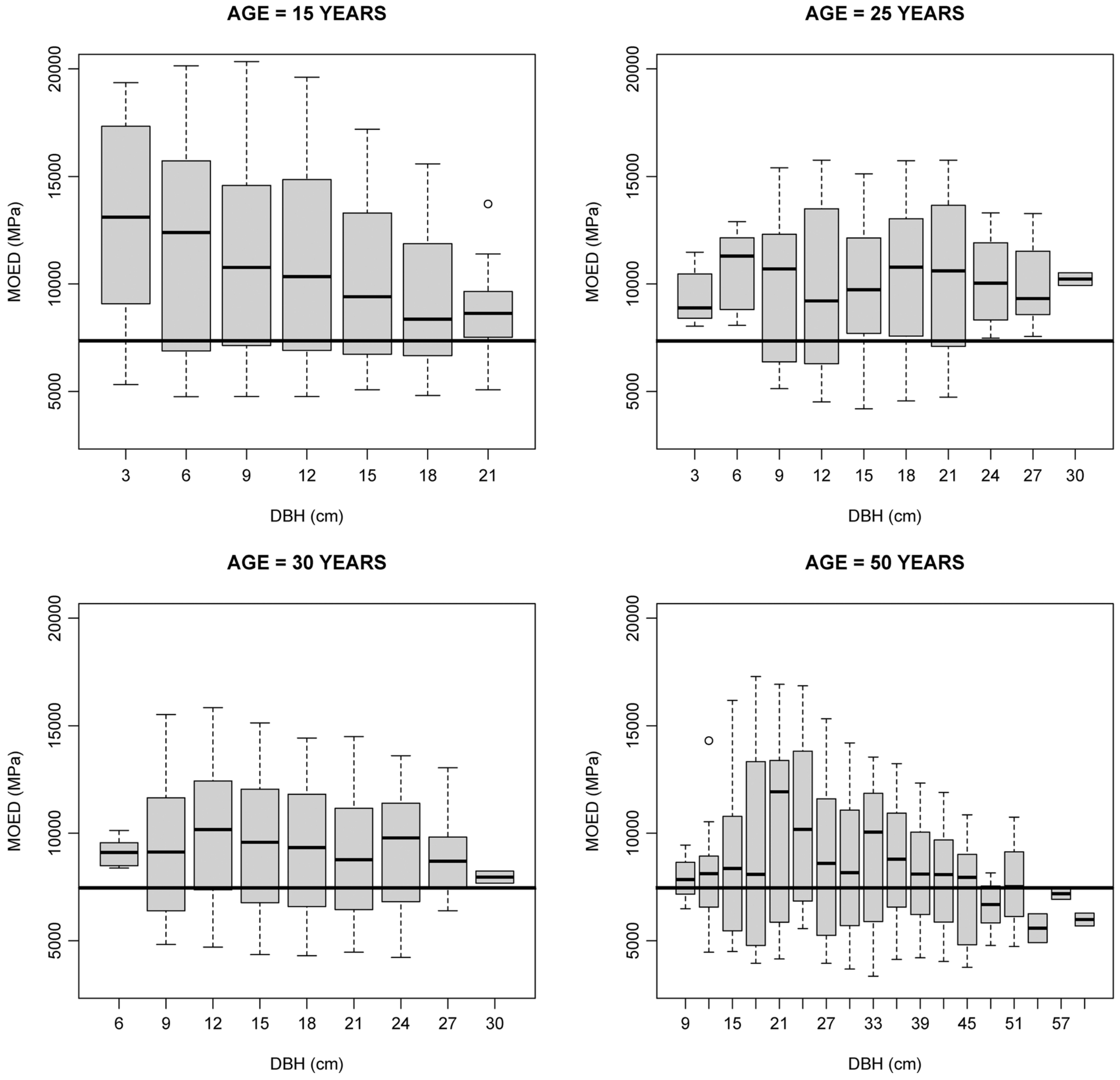


Fig. 8 Variation of the MOEd values as DBH increase for the stands at 15, 25, 30, and 50 years. In the boxplots, the horizontal lines indicate the median values; the lower limit corresponds to the value of the first quartile (Q1) of the distribution and the upper limit to the third quartile (Q3); the vertical lines (whiskers) delimit the intervals in which the lower values of Q1 (in the lower part) and the greater values of Q2 (in the upper part) are positioned. In each graph, the horizontal lines represent the minimum MOEd value recommended (7200 MPa; see Detter et al. 2008)

at the culmination age (about 18 m^3 ha^{-1} year^{-1} at 25 years) is higher than that reported in other studies done in different environmental conditions: 11 m^3 ha^{-1} year^{-1} at 40 years in the Dean Forest in Southern England for the best qualities (Everard and Christie 1995) and 16 m^3 ha^{-1} year^{-1} at 30 years in France (Bourgeois et al. 2004).

The present study reports model results aligned with previous observations on chestnut coppices. With reference to the basal area, values of 25 m^2 ha^{-1} were observed for 11-year-old chestnut coppices in Tuscany (Manetti et al. 2016), while values ranging from 18 to 42 m^2 ha^{-1} were recorded for 6–22-year-old chestnut coppices in Lazio (Mattioli et al. 2016), both considering Central Italy. Again, results of the present study are consistent with previous research on chestnut coppices and, thus, the model exercise may help implementation processes and scaling procedures in different environmental setting.

The variability in local conditions across different geographical contexts, due to specific environmental factors and different management options, may affect model results. However, the homogeneous environmental setting and land-use history across plots throughout the chronosequence clearly indicated high reliability of the model predictions and simulation of the coppice productivity. Indeed, shoot heights reached 22 m in 50-year-old coppice stands, thus potentially ensuring wood-based products of high economic value. Particularly, the height–diameter curves revealed that, during the earlier stages after coppicing, shoots might show a considerable height increment. In fact, a relevant height–diameter curve differentiation was observed at these growth stages, probably due to the strong competition for light between shoots occurring on each single stool. When the coppice reached an age of 15–20 years and beyond, the height–diameter curve flattened, indicating a high level of spatial competition between shoots (Marziliano et al. 2013, 2019). At these ages, the competition is mainly affected by diametrical differentiation rather than by hypsometric variation, shoots growing more in diameter than in height.

Chestnut is considered the tree species with the highest capacity to provide multiple goods and services among Mediterranean forest species (Giannini et al. 2014), producing a variety of assortments other than firewood, even when it is managed as coppice stand. In this study, we highlighted the great potential of chestnut for producing different wood-based products when the rotation period of coppice stands was extended. In this context, although the organic layer and the mineral soil, as a large carbon stock in forest ecosystems, were not accounted for in this study, implications for carbon sinks and mitigation purposes are also important.

On the other hand, for the same assortments, wood quality traits might significantly vary, depending on the length of rotation periods. In fact, MOEd values revealed a negative trend as stand age increased (from to 25 to 50 years) while such a trend was positive at stand ages growing from 15 to 25 years. Moreover, a negative trend of MOEd values was observed as DBH increased, both in C15 and C50. According to Detter et al. (2008), chestnut wood-based products can be considered of good quality when the MOEd is not lower than 7200 MPa. However, in C15 and C50, we recorded MOEd values lower than this threshold value, for DBH higher than 18 cm (7000–7100 MPa) and 45 cm (5587–7100 MPa), respectively. Therefore, almost all large poles (assortments of the greatest size) attainable in C15 and most of beams and boards (assortments with considerable size) obtainable in C50 did not have the minimum quality requirements for being classified as adequate. Quality wood could, however, be produced at relatively high stand age and DBH, when chestnut coppices grow in good site conditions and with appropriate silvicultural treatments (Manetti et al. 2020).

These results demonstrate that the advanced shoot ages, but also the high growth rates of chestnut coppices, negatively affected the wood quality. For this reason, the dynamics of stem radial increments might induce the production of a less-stiff mature wood, resulting in a significant loss of wood quality. Romagnoli et al. (2014) obtained similar results and observed, in coppice stands with age higher than 25–30 years, a decrease in the mechanical performance of chestnut wood near the threshold DBH of 35 cm. Therefore, to maximize and balance wood quality and stand productivity, coppicing in this Mediterranean context should be rethought in terms of strengths and weaknesses of the system, considering not only the shoot age, but also the shoot DBH at harvest (Genet et al. 2013), as well as shoot height and basal area (Marini et al. 2021).

We observed that, in the present conditions, when the rotation cycle ranged between 25 and 30 years, wood-based products of high quality could be obtained, as well as a variety of assortments. Nevertheless, 20–30-year-old chestnut stands, growing on favourable sites and properly managed, could be considered young and still have high growth rates (Cutini 2001; Conedera et al. 2004; Manetti et al. 2009). Similarly, we observed MAI equal to 13.3 m^3 ha^{-1} $year^{-1}$ at 26 years (year of culmination). Amorini and Manetti (1997) found that wood of good quality could be produced with rotation periods ranging between 30 and 50 years and with 2–4 medium-intensity thinning operations. It must be pointed out that, in many areas of Italy, common rotation periods range between 10 and 15 years (Ciancio et al. 2004). Such short rotation periods appear to be inadequate to ensure chestnut wood of good quality (Manetti et al. 2006). Indeed, only assortments of small size and poor quality were obtained in the present study, when short rotation periods were considered. Nowadays, commercial operators and the timber market in general often require assortments of good wood quality, obtainable from the chestnut coppices by lengthening to some extent the rotation period currently adopted in most chestnut coppices (Angelini et al. 2013; Mattioli et al. 2016; Manetti et al. 2017). Extended rotation periods (in the range of 30–50 years) would also positively affect the provisioning of ecosystem services related to environmental issues (Gondard and Romane 2005; Gondard et al. 2006). However, the lengthening of rotation periods without thinning might cause considerable competition stress, irregular radial growth and, consequently, ring shake risk.

Thinning would allow a greater average DBH of shoots to be obtained at earlier growth stages, with consequent differentiation of assortments (Mattioli et al. 2016). However, many studies have shown that marked increments in stem diameter after intensive thinning, especially if carried out late, might induce less-stiff mature wood, resulting in a significant loss of wood quality at high DBH (Zhang 1995; Ikeda and Kino 2000; Wang et al. 2003; Štefancík et al. 2018). Marini et al. (2021) found a negative correlation between wood density and dominant height and diametric growth of chestnut coppice stands, and this was associated to tree ring width. Should stand density, i.e., the number of shoots per ha, be a factor affecting positively wood density and the related mechanical properties, the application of low to moderate thinning might favour the formation of wood-based products of good quality in these chestnut coppices. However, caution is needed to avoid overextrapolation of these results. Indeed, stand can be of such poor quality that the obtained wood products have no commercial value. Therefore, particular care should be considered when thinning is planned and executed, avoiding both strong intensity and late thinning, and monitoring wood quality. By modifying competition (the number of shoots per ha) and, thus, shoot diameter growth and stand basal area, thinning might increase the risk of ring shake (Fonti et al. 2002; Becagli et al. 2006; Romagnoli et al. 2014). All these negative effects would limit the use of chestnut wood for the most valuable assortments, i.e., those usable as structural elements (Fonti et al. 2002).

Conclusions

The chronosequence approach was proved useful to show the effect of varying rotation periods on wood quality of homogeneous (except for age) chestnut coppice stands. A moderately negative correlation between shoot age and wood quality was observed. We also documented that innovative and non-destructive methods might produce wood technological indicators, i.e., MOEd, which relate shoot age and DBH to wood quality. These indicators may serve as tools for monitoring wood quality over time and provide valuable information that can aid decision-making in forest management.

Effective predictors for assessing wood quality may support stakeholders to develop management strategies that require control over stand characteristics. Nowadays, in many regions of Italy, the rotation period for chestnut coppices lasts 15–18 years. As such, harvest may take place too early compared to the economically and ecologically optimum rotation length for balancing forest profitability and carbon sequestration. Therefore, lengthening the rotation period to 25–30 years would benefit both productivity and quality of wood as well as landscape conservation and climate matter in Mediterranean mountain systems.

Author's contributions PAM and FL designed the study and analysed the data. MM and AL collected the data in the field. PAM, RT, and FL wrote the manuscript. All authors discussed the results and reviewed the manuscript.

Funding Open access funding provided by Università degli Studi Mediterranea di Reggio Calabria within the CRUI-CARE Agreement. This work was supported by the Mediterranean University of Reggio Calabria.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Competing interests The authors declare no competing interests.

References

Aide TM, Zimmerman JK, Pascarella JB, Rivera L, Marcano Vega H (2000) Forest regeneration in a chronosequence of tropical abandoned pastures: implications for restoration ecology. Restor Ecol 8:328–338

Amorini E, Manetti MC (1997) Le 'fustaie da legno' di castagno del Monte Amiata. Ann Ist Sper Selv 28:53–61

Angelini A, Mattioli W, Merlini P, Corona P, Portoghesi L (2013) Empirical modelling of chestnut coppice yield for Cimini and Vicani mountains (Central Italy). Ann Silvic Res 37: 7–12. https://doi.org/10.12899/ASR-749

Becagli C, Amorini E, Manetti MC (2006) Incidenza della cipollatura in popolamenti cedui di castagno da legno del Monte Amiata. Annali Dell'istituto Sperimentale per La Selvicoltura 33:245–256

Bernetti G (1980) Auxometria dei boschi cedui italiani. L'italia Forestale e Montana 1:1–24

Bourgeois C, Sevrin E, Lemaire J (2004) Le châtaignier: un arbre, un bois, 2nd, revised. Institut pour le dèveloppement forestier, Paris, p 347

Bowditch E, Santopuoli G, Binder F, del Río M, La Porta N, Kluvankova T, Lesinski J, Motta R, Maciej P, Panzacchi P, Pretzsch H, Temperli C, Tonon G, Smith M, Velikova V, Weatherall A, Tognetti R (2020) What is Climate-Smart Forestry? A definition from a multinational collaborative process focused on mountain regions of Europe. Ecosyst Serv 43:101113

Buckley GP (1992) Ecology and management of coppice woodlands. Chapman & Hall, London

Cabrera BM, Ochoa (1997) Tablas de producción de castaño (*Castanea sativa* Mill.) tratado en monte bajo, en Asturias. In Puertas F, M Rivas M eds. II Congreso Forestal Español-Irati 97. Pamplona, Spain, pp 131–136

Ciancio O, Garfì V, Iovino F, Menguzzato G, Nicolaci A (2004) I cedui di castagno in Calabria: caratteristiche colturali, produttività e assortimenti ritraibili. L'italia Forestale e Montana 59:1–14

Clutter JL, Fortson JC, Pienaar LV, Brister GH, Bailey RL (1983) Timber management: a quantitative approach. John Wiley and Sons, Inc., New York, p 333

Conedera M, Manetti MC, Giudici F, Amorini E (2004) Distribution and economic potential of the Sweet Chestnut (*Castanea sativa* Mill.) in Europe. Ecol Mediterr 30:179–193

Conedera M, Tinner W, Krebs P, de Rigo D, Caudullo G (2016) *Castanea sativa* in Europe: distribution, habitat, usage and threats. In: San-Miguel-Ayanz J, de Rigo D, Caudullo G, Houston Durrant T, Mauri A (Eds.), European Atlas of Forest Tree Species. Publ. Off. EU, Luxembourg, pp. e0125e0+

Cutini A (2001) New management options in chestnut coppices: an evaluation on ecological basis. For Ecol Manag 141:165–174

Cutini A, Ferretti M, Bertini G, Brunialti G, Bagella S, Chianucci F, Fabbio G, Fratini R, Riccioli F, Caddeo C, Calderisi M, Ciucchi B, Corradini S, Cristofolini F, Cristofori A, Di Salvatore U, Ferrara C, Landi FL, , Marchino L, Patteri G, Piovosi M, Roggero PP, Seddaiu G, Gottardini E (2021) Testing an expanded set of sustainable forest management indicators in Mediterranean coppice area. Ecol Indic 130:108040. https://doi.org/10.1016/j.ecolind.2021.108040

Detter A, Cowell C, McKeown L, Howard P (2008) Evaluation of current rigging and dismantling practices used in arboriculture. Research Report RR668. Health and Safety Executive (HSE) and Forestry Commission (FC), UK, 370.

Divos F (2010) Acoustic tools for seedling, tree and log selection. In: Proceedings of the final conference of COST Action E53 the future of quality control for wood and wood products, Edinburgh, UK, 4–7 May 2010; p. 5.

Elorrieta J (1949) El castaño en España. Instituto Forestal de Investigaciones Agrarias (I.F.I.E.). Número 48. Madrid, España. Ministerio de Agricultura. 303 p.

Esteban E, Spinelli R, Aminti G, Laina RL, Vicens I (2018) Productivity, efficiency and environmental effects of whole-tree harvesting in spanish coppice stands using a drive-to-tree disc Saw Feller-Buncher. Croat J for Eng 39:163–172

Everard J, Christie JM (1995) Sweet chestnut: silviculture, timber quality and yield in the Forest of Dean. Forestry 68: 133–144. Fabbio G (2016) Coppice forests, or the changeable aspect of things, a review. Ann Silvicult Res 40:108–132

Fabbio G (2016) Coppice forests, or the changeable aspect of things, a review. Ann Silvicult Res 40(29): 108–132

FAO (2014) World reference base for soil resources. International Soil Classification System for Naming Soils and Creating Legends for Soil Maps. World Soil Resources Reports No. 106; Roma, Italy.

Fonti P, Macchioni N, Thibaut B (2002) Ring shake in chestnut (*Castanea sativa* Mill.): state of the art. Ann For Sci 59:129–140. https://doi.org/10.1051/forest:2002007

Genet A, Auty D, Achim A, Bernier M, Pothier D, Cogliastro A (2013) Consequences of faster growth for wood density in northern red oak (*Quercus rubra* Liebl.). Forestry 86:99–110

Giannini R, Maltoni A, Mariotti B, Paffetti D, Tani A, Travaglini D (2014) Valorizzazione della produzione legnosa dei boschi di castagno. L'italia Forestale e Montana 69:307–317

Gondard H, Romane F (2005) Long-term evolution of understorey plant species composition after logging in chestnut coppice stands (Cevennes Mountain, Southern France). Ann Sci For 62:333–342

Gondard H, Romane F, Santa Regina I, Leonardi S (2006) Forest management and plant species diversity in chestnut stands of three Mediterranean areas. Biodivers Conserv 15:1129–1142

Greco S, Infusino M, Ienco A, Scalercio S (2018) How different management regimes of chestnut forests affect diversity and abundance of moth communities? Ann Silvic Res 42:59–67. https://doi.org/10.12899/asr-1503

Guntekin G, Emiroglu ZG, Yolmaz T (2013) Prediction of bending properties for Turkish red pine (*Pinus brutia* Ten.) Lumber using stress wave method. BioResources 8:231–237

Hedde M, Aubert M, Decaëns T, Bureau F (2008) Dynamics of soil carbon in a beechwood chronosequence forest. For Ecol Manag 255:193–202

Hédl R, Kopecky M, Komàrek J (2010) Half a century of succession in a temperate oakwood: from speciesrich community to mesic forest. Divers Distrib 16:267–276

Ikeda K, Kino N (2000) Quality evaluation of standing trees by a stress-wave propagation method and its application I. Seasonal changes of moisture contents of sugi standing trees and evaluation with stress-wave propagation velocity. Mokuzai Gakkaishi 46:181–188

INFC (2005) Le stime di superficie 2005. Inventario Nazionale delle Foreste e dei Serbatoi Forestali di Carbonio. MiPAF, Corpo Forestale dello Stato, CRA-ISAFA, Trento.

Johnson EA, Miyanishi K (2008) Testing the assumptions of chronosequences in succession. Ecol Lett 11:419–431

Kelly C, Ferrara A, Wilson GA, Ripullone F, Nolè A, Harmer N, Salvatic L (2015) Community resilience and land degradation in forest and shrubland socio-ecological systems: evidence from Gorgoglione, Basilicata, Italy. Land Use Policy 46:11–20

Kneifl MM, Kadavý J, Knott R, Adamec Z, Drápela K (2015) An inventory of tree and stand growth empirical modelling approaches with potential application in coppice forestry (A review). Acta Universitatis Agriculturae Et Silviculturae Mendelianae Brunensis 63:1789–1801

Lanuv (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen), (2007) Niederwälder in Nordrhein-Westfalen. Galunder-Verlag, Nümbrecht-Elsenroth

Lemaire J (2008) Estimer la potentialité de son taillis de châtaignier et adapter les éclaircies. Forêt Enterprise 179:14–17

Manetti MC, Amorini E, Becagli C (2006) New silvicultural models to improve functionality of chestnut stands. Adv Hortic Sci 1:65–69

Manetti MC, Amorini E, Becagli C, Pelleri F, Pividori M, Schleppi P, Zingg A, Conedera M (2009) Quality wood production from chestnut (*Castanea sativa* Mill.) coppice forests - Comparison between different silvicultural approaches. In: Proceeding of the "1st European Congress on Chestnut" (Bounous G, Becarro G eds). Acta Horticulturae 866: 683–692.

Manetti MC, Becagli C, Sansone D, Pelleri F (2016) Tree-oriented silviculture: a new approach for coppice stands. iForest 9: 791–800

Manetti MC, Becagli C, Carbone F, Corona P, Giannini T, Pelleri F, Romano R (2017) Linee guida per la selvicoltura dei cedui di castagno. Rete Rurale Nazionale, Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Roma, ISBN: 9788899595579. 50 p.

Manetti MC, Marcolin E, Pividori M, Zanuttini R, Conedera M (2020) Coppice woodlands and chestnut wood technology. In: Beccaro G, Alma A, Bounous G, Gomes-Laranjo J (eds), The chestnut handbook. Crop and forest management, pp. 275–295. https://doi.org/10.1201/9780429445606-10.

Marcolin E, Manetti MC, Pelleri F, Conedera M, Pezzatti GB, Lingua E, Pividori M (2020) Seed regeneration of sweet chestnut (*Castanea sativa* Miller) under different coppicing approaches. For Ecol Manag 472:118273

Marini F, Manetti MC, Corona P, Portoghesi L, Viniguerra V, Tamantini S, Kuzminsky E, Zikeli F, Romagnoli M (2021) Influence of forest stand characteristics on physical, mechanical properties and chemistry of chestnut wood. Sci Rep 11:1549. https://doi.org/10.1038/s41598-020-80558-w

Marziliano PA, Lafortezza R, Colangelo G, Davies C, Sanesi G (2013) Structural diversity and height growth models in urban forest plantations: a case-study in northern Italy. Urban for Urban Green 12:246–254

Marziliano PA, Tognetti R, Lombardi F (2019) Is tree age or tree size reducing height increment in *Abies alba* Mill. at its southernmost distribution limit? Ann For Sci 76.

Mattioli W, Mancini LD, Portoghesi L, Corona P (2016) Biodiversity conservation and forest management: the case of the sweet chestnut coppice stands in Central Italy. Plant Biosyst 150:592–600. https://doi.org/10.1080/11263504.2015.1054448

Menéndez-Miguélez M, Canga E, Álvarez-Álvarez P, Majada J (2014) Stem taper functions for sweet chestnut (*Castanea sativa* Mill.) coppice stands in northwest Spain. Ann for Sci 71:761–770

Menéndez-Miguélez M, Álvarez-Álvarez P, Majada J, Canga E (2015) Effects of soil nutrients and environmental factors on site productivity in *Castanea sativa* Mill. coppice stands in NW Spain. New Forest 46:217–233

Menéndez-Miguélez M, Álvarez-Álvarez P, Majada J, Canga E (2016) Management tools for *Castanea sativa* coppice stands in northwestern Spain. Bosque 37:119–133

Moscatelli M, Pettenella D, Spinelli R (2007) Produttività e costi della lavorazione meccanizzata dei cedui di Castagno in ambiente appenninico. Forest@ 4, 51–62.

Parisi F, Lombardi F, Marziliano PA, Russo D, De Cristofaro A, Marchetti M, Tognetti R (2020) Diversity of saproxylic beetle communities in chestnut agroforestry systems. iForest 13: 456–465. https://doi.org/10.3832/ifor3478-013 [online 2020–10–07]

Pastorella F, Giacovelli G, Maesano M, Paletto A, Vivona S, Veltri A, Pellicone G, Scarascia Mugnozza G (2016) Social perception of forest multifunctionality in southern Italy: the case of Calabria Region. J For Sci 62:366–379

Patricio MS, Monteiro ML, Nunes LF, Mesquita S, Beito S, Casado J, Guerra H (2005) Management models evaluation of a *Castanea sativa* coppice in the northeast of Portugal. In: Proceedings of the Third International Chestnut (Ed.: Abreu CG, Rosa E, Monteiro AA). Acta Horticulturae 693: 721–725.

Pawson SM, Brockerhoff EG, Didham RK (2009) Native forest generalists dominate carabid assemblages along a stand-age chronosequence in anexotic *Pinus radiata* plantation. For Ecol Manag 258S:S108–S116

Pickett STA (1989) Space-for-time substitutions as an alternative to longterm studies. In: Likens GE (ed) Longterm studies in ecology. Springer, New York.

Pretzsch H (2009) Forest dynamics, growth and yield. Springer Verlag, Berlin

R Core Team (2019) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. [online] URL: http://www.r-project.org

Romagnoli M, Cavalli D, Spina S (2014) Wood quality of chestnut: relationships between ring width, specific gravity and physical and mechanical properties. Bioresources 9:1132–1147. https://doi.org/10.15376/biores.9.1.1132-1147

Russo D, Marziliano PA, Macri G, Proto AR, Zimbalatti G, Lombardi F (2019) Does thinning intensity affect wood quality? An analysis of Calabrian Pine in Southern Italy using a non-destructive acoustic method. Forests 30:1–16

Russo D, Marziliano PA, Macrì G, Zimbalatti G, Tognetti R, Lombardi F (2020) Tree growth and wood quality in pure vs. mixed-species stands of European beech and Calabrian pine in Mediterranean mountain forests. Forests 11: 6. https://doi.org/10.3390/f11010006.

Rydberg D (2000) Initial sprouting, growth and mortality of European aspen and birch after selective coppicing in central Sweden. For Ecol Manag 130:27–35

Salisbury E (1952) Downs and Dunes: Their Plant Life and Environment. G. Bell & Sons Ltd., London, UK, p 1952

Scarascia-Mugnozza G, Oswald H, Piussi P, Radoglou K (2000) Forests of the Mediterranean region: gaps in knowledge and research needs. For Ecol Manag 132:97–109

Scherzinger W (1996) Naturschutz im Wald: Qualitätsziele einer dynamischen Waldentwicklung. Praktischer Naturschutz. — Stuttgart (Verlag Eugen Ulmer). 447 S., 51 Farbabb., 119 s/w-Abb., 36 Tab. ISBN 3-8001-3356-3.

Skovsgaard JP, Vanclay JK (2013) Forest site productivity: a review of spatial and temporal variability in natural site conditions. Forestry 86:305–315. https://doi.org/10.1093/forestry/cpt010

Štefancík I, Vacek Z, Sharma RP, Vacek S, Rösslová M (2018) Effect of thinning regimes on growth and development of crop trees in *Fagus sylvatica* stands of Central Europe over fifty years. Dendrobiology 79:141–155

Tabacchi G, Di Cosmo L, Gasparini P (2011) Aboveground tree volume and phytomass prediction equations for forest species in Italy. Eur J For Res 130:911–934

Teder M, Pilt K, Miljan M, Lainurm M, Kruuda R (2011) Overview of some non-destructive methods for in-situ assessment of structural timber. In Proceedings of the 3rd International Conference Civil Engineering, Jelgava, Latvia, 12–13 May 2011.

Vanclay JK (1994) Modelling forest growth and yield. CAB International, Wallingford

Vanninen P, Ylitalo H, Sievanen R, Makela A (1996) Effect of age and site quality on the distribution of biomass in Scots pine (*Pinus sylvestris* L.). Trees 10:231–238

Von Gadow K, Hui G (1999) Modelling forest development. Kluwer, Dordrecht

Walker et al (2010) The use of chronosequences in studies of ecological succession and soil development. J Ecol 98:725–736

Wang X, Ross RJ, Punches J, Barbour RJ, Forsman JW, Erickson JR (2003) Evaluation of small-diameter timber for valued-added manufacturing—a stress wave approach. In Proceedings of the II International Precision Forestry Symposium; Institute of Forest Resources, University of Washington: Seattle, WA, USA, pp. 91–96.

Weiskittel AR, Hann DW, Kershaw JA, Vanclay JK (2011) Forest growth and yield modeling. Wiley, Chichester

Wessels CB, Malan FS, Rypstra T (2011) A review of measurement methods used on standing trees for the prediction of some mechanical properties of timber. Eur J For Res 130:881–893

Zhang SY (1995) Effect of growth rate on wood specific gravity and selected mechanical properties in individual species from distinct wood categories. Wood Sci Technol 29:451–465

Zlatanov T, Schleppi P, Velichkov I, Hinkov G, Georgieva M, Eggertsson O, Zlatanova M, Harald V (2013) Structural diversity of abandoned chestnut (*Castanea sativa* Mill.) dominated forests: implications for forest management. For Ecol Manag 291:326–335. https://doi.org/10.1016/j.foreco.2012.11.015

Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. Methods Ecol Evol 1:3–14