Tree-ring response of two relict species *Fraxinus angustifolia* Vahl and *Quercus robur* L. to drought at San Rossore (Pisa, Italy)

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1 Summary

The future climatic scenarios suggest a disproportionately strong increase of temperature and aridity in the Mediterranean region. Therefore, understanding ecosystem's response to drought in the region has been a subject to many researches in diverse fields. The study of tree-rings has been a reliable tool for studies on drought, because an accurate date is registered from the formation of the rings in relationship with the climatic event. The majority of existing research focuses on xeric sites. However, studies of trees growing at mesic sites have indicated that the effect of drought stress on growth dynamics is stronger in mesic than in xeric stands. Studies in mesic sites are rare because of their limited geographic distribution and strong anthropogenic interference.

At four sites of the San Rossore forests (Pisa, Italy) I have found relict species growing in mesic sites that could suit this study demands. I have collected cores from *Quercus robur* L., *Fraxinus angustifolia* Vahl growing in interdunes and *Pinus pinea* L. growing at the dunes. The growth of these species associated with drought has not been deeply explored yet. I have analyzed the tree-rings interactions with mean monthly precipitation, mean monthly maximum temperature and mean monthly minimum temperature since 1922.

The results have shown that *Quercus*, *Fraxinus* and *Pinus* are more limited by precipitation than by temperature at San Rossore. Precipitation during the winter seems to be the driver for tree growth of *Fraxinus* and *Quercus*, while the driver for tree growth of *Pinus* might be precipitation during late spring-summer. Contrary to the expectations, *Pinus* grows more narrow rings than *Quercus* and *Fraxinus*, but they were not related to strong drought events. In the interdunes, *Quercus* grows narrow rings more often than *Fraxinus*, when strong drought events occur. *Quercus* is strongly dependent in continuous water availability while *Fraxinus* seems to be more adapted to the oscillations of the water table. Growth synchronicity among same species is better than growth synchronicity between different species. This shows that species have different ecological niches. Therefore each species could have benefited differently from site conditions and microclimate and thus have avoided the stress from extreme climatic conditions. In a long term perspective, with increasing temperatures and decreasing precipitation in the future, *Quercus robur* would probably be the first to suffer from extreme drought events since it is highly dependent on continuous water availability, allowing species such as *Fraxinus angustifolia* to spread and possibly dominate mesic sites in the Mediterranean.

2 Introduction

Forest ecosystems and the ecological processes occurring in them are strongly dependent on the climatic conditions, events and trends, in which they develop. Climatic conditions in many regions of the world are characterized by high variability, and forests have been adapting to changes over time. Several studies have shown the ample capacity of temperate and tropical forests to cope with climate change (e.g., Cook et al. 2001). However, recently, an unprecedented increase in temperature has been recorded in the northern hemisphere, and for the future, dramatic climatic changes are predicted in several regions of the world.

The Mediterranean region is expected to experience disproportionately strong increases of temperature and aridity (Pañuelas 1996, IPCC 2007, Valladares 2008) in the future. This will possibly result in a higher frequency and severity of droughts than in the last century. Garcia-Ruiz et al. (2011) in their review of long-term dendrochronological studies from the Mediterranean region, show that drought events during the second half of the 20th century were the most severe in the last 500 years.

The impact of drought on different tree species in the Mediterranean is not well known. Depending on the response of each species to increasing drought frequency and severity, species composition of Mediterranean ecosystems will change accordingly. Some species will disappear and others become dominant. Examining tree rings can be a reliable tool to understand the effect of past drought events on different tree species, their growth and physiology. The exact dating of each ring gives perfect annual resolution allowing a precise study of the impact of disturbance events.

The Mediterranean region is commonly defined by its climate. Tree species face two different types of stress; summer drought and winter low temperature (Cherubini et al. 2003). Both temperature and precipitation strongly vary throughout the region, but while summer high temperatures affect the Mediterranean as a whole, mean annual precipitation differs from region to region very strongly. In the Italian peninsula e.g., annual precipitation ranges from 100 to 2000 mm, which means that some regions of the Mediterranean experience desert-like precipitation (Fady and Médail 2004). Tree species have therefore developed ways to cope with summer drought; a drought-avoidance strategy (e.g., by shedding their leaves in summer) or a drought-tolerance strategy, continuing their growth during the dry season (de Lillis & Fontanella, 1992).

Although climatic conditions, being harsh, impose severe limitations to the survival of species in the Mediterranean, the region is nevertheless characterized by a high floristic diversity of forests (Fady and Médail 2004). During the last glaciation, the geographic distribution of vegetation changed strongly. Temperate forest ecosystems survived in glacial refugia at mid-to-high latitudes as on the Tyrrhenian coast of central Italy (Petit et al. 2003, Lucchi 2008, Valladares 2008). The migration of these species had a strong effect on the current spatial distribution of woody species and the main forest ecosystems around the Mediterranean and in Europe. As a consequence, the present day tree flora of the Mediterranean Basin is made up of very resilient taxa that have already experienced many abrupt and intense climate changes in the past (Bennett et al. 1991, Petit et al. 2003), and very vulnerable taxa that are climatically isolated and restricted to places where humidity allows them to survive until now.

Quercus robur and *Fraxinus angustifolia* are among the refugia species that persisted in such environments so they are not autochthones of the Mediterranean. The growth of these species associated with drought hasn’t been explored in detail. Firstly because anthropogenic activities left few forest patches preserved in the Mediterranean and suitable research sites are hard to be found. Secondly, because these trees are associated with mesic sites and the impact of drought is expected to be less evident than on xeric stands. However, ecological studies of trees growing at mesic (temperate and Mediterranean) sites (Glenz et al. 2006, Rodríguez-González et al. 2010, Pasho et al. 2012, Rivaes et al. 2012) have shown that warming related drought stress might affect growth dynamics more in mesic than in xeric stands.

Dendroecological studies have been a helpful tool to understand the response of tree species growing at mesic sites (e.g., Cater 2011). Therefore, a dendroecological study of *Quercus* sp. and *Fraxinus* sp. in the Mediterranean would significantly contribute to the understanding of the behavior of these species during extreme climatic events.

2.1 San Rossore forest

Dendroclimatological research in a Mediterranean country is time consuming.
The old forest stands must first be found which is very hard (…) Richter 1988.

San Rossore’s forest area, close to Pisa, Italy (43°43’N, and 10°19’E), lies on the Tyrrhenian coastline. It is part of the San Rossore, Migliarino and Massaciuccoli Regional Park which was established in 1979. However, the area was already known since the Middle Ages as a vast forested site (Mazzatti 1994).

Before the 1500’s, the region, characterized by xerophilous and meso-hygrophilous hardwood forests (e.g., oaks, ashes refugia species) was mainly used for forestry, grazing activities and leisure of the noble families, e.g., hunting (Fig. 1). Later on, when Pisa fell under Florence rules, the territory was rearranged and managed by the Salviatis, a Florentine family (Agnoletti 2005). At this epoch, the settlers intervened on the protection of the land by establishing regulations limiting the access to all natural resources. Only logging (which received forestry control) wasn’t fully erased because oak was used for the naval industry (Mori et al. 2007).

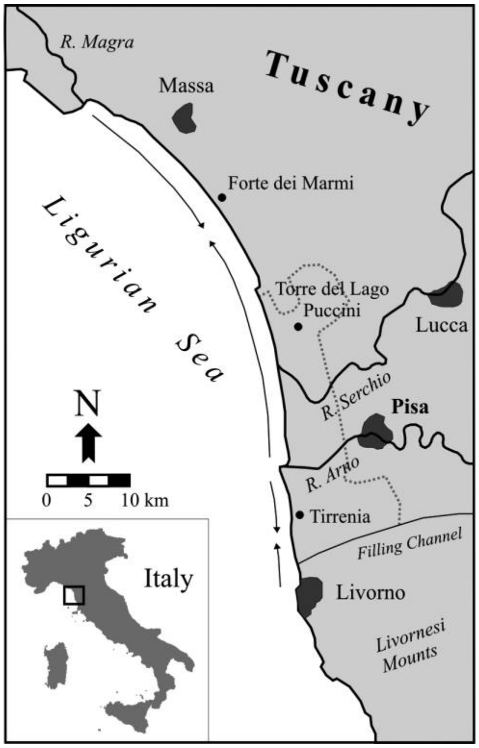


Fig. 1: Localization of San Rossore (light dotted grey line. Source: Bertoni & Sarti 2011

The first reforestation action in San Rossore happened at the end of the 1800's. Stone pine was introduced and the drainage system in the property was rearranged (Mazzatti, 1994). In the 20th century (until 1960's) the park was the largest center of pine kernel production in Italy (Peruzzi et al. 1998).

Fig. 2: The Hunt in the Forest by Paolo Uccello 1470. The painting depicts a hunt on a wetland surrounded by an Oak forest allegedly at the San Rossore area. Source: photo by Andrea Bertacchi.

The San Rossore forest offers various alternatives for the study in xeric and mesic sites. It is one of the remaining patch forests in the Mediterranean where refugia species have settled and where *Fraxinus* and *Quercus* are found.

2.1.1 The Climate

The climate at the forest is Mediterranean sub-humid, characterized by a long dry season; summer precipitation around 100 mm and a monthly average of the warmest month at 23°C.

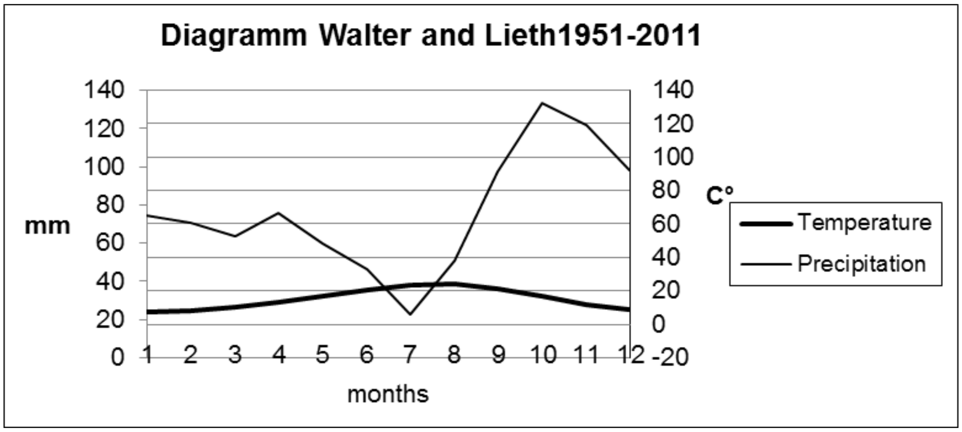


Fig. 3: Average yearly temperature (°C) and average yearly precipitation (mm) in Pisa from 1951-2011. Source: Facoltà di Agraria Pisa.

The wind yearly average frequency comes with the highest speed going over 50 knots (Sarti et al., 2008). The average yearly temperature is 14.8°C and rainfall is 900 mm.

2.1.2 The Geology

The San Rossore area falls within the tectonic system called Versiliese-Pisana trench. This subsiding area, where a lagoon was once fed by the Arno and Serchio rivers, has been transformed into a deposit with enormous amounts of sediments (Perfetti, 2010). The regressions and transgression of the sea level during the Quaternary formed an alternation of sandy and silt-clay sediments characteristic of the beaches and dunes at the coast of Tuscany.

The changes in landscape during the last centuries are illustrated by comparisons of historical maps with the current situation. One of the first maps, drawn by Leonardo Da Vinci in 1503, shows that the distance from Pisa to the Tyrrhenian coastline was of 7.0 km (Fig. 4) as for today this distance is about 10 km from which 5.0 km is the extension of the San Rossore forest area from the coast to its inland borders (Fig. 1).

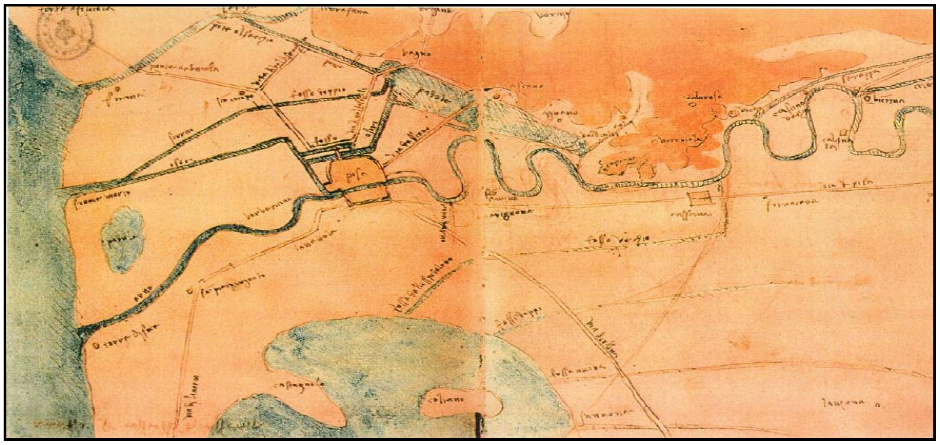


Fig. 4: Leonardo Da Vinci, Corografia del Valdarno da Pontedera al mare, 1503, Madrid, Biblioteca Nacional, Ms. 8937 II cc. 52v 53r. Source: Bottai and Barsotti 1994.

2.1.2.1 The Dune-Interdune system

At this Pisan flood plain, the action of the winds, ocean currents and tides, explains the increasingly rapid progress of the coast. During the Roman times, a port existed (Bottai and Barsotti 1994) where forests in system of dunes and interdunes stand nowadays (Fig. 5). Under favorable conditions, when dunes begin to form, a consolidated sandbank builds up in front of the dunes. The new growing beach tends to join the pre-existing shore and causes a sheet of water to accumulate between the new dune and the old coastline. Finally, further sediments are deposited closing any connections with the sea and a proper retrodunal lagoon is formed (Barducci et al., 2009).

There is a gradient in soil texture (sand, silt and clay) in a transect moving from the coast to inland (Sarti et al., 2008). Soils are sandy on the coast and more porous. Finer textures (silt-clay) and higher pH values can be found inland, where soils are less porous. The different amount of organic matter of the forest vegetation also affects the characteristics of the soil; conifers and broad-leaved trees differently influence the pedogenesis.

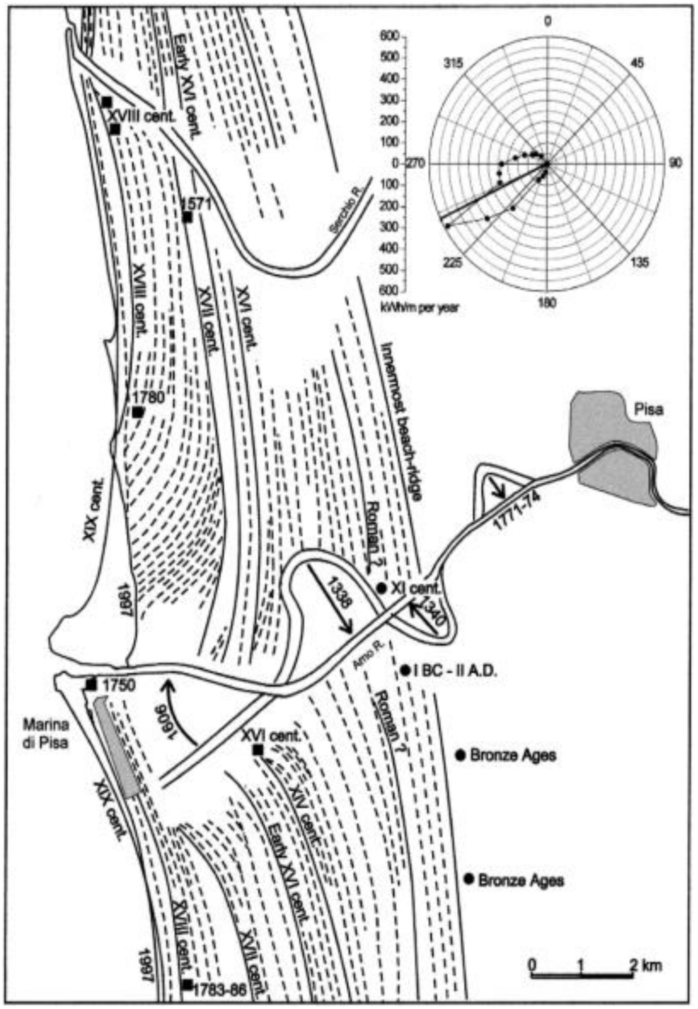


Fig. 5: Development of the dune-interdune system in San Rossore. Source: Sarti et al. 2008.

2.1.3 The hydrology

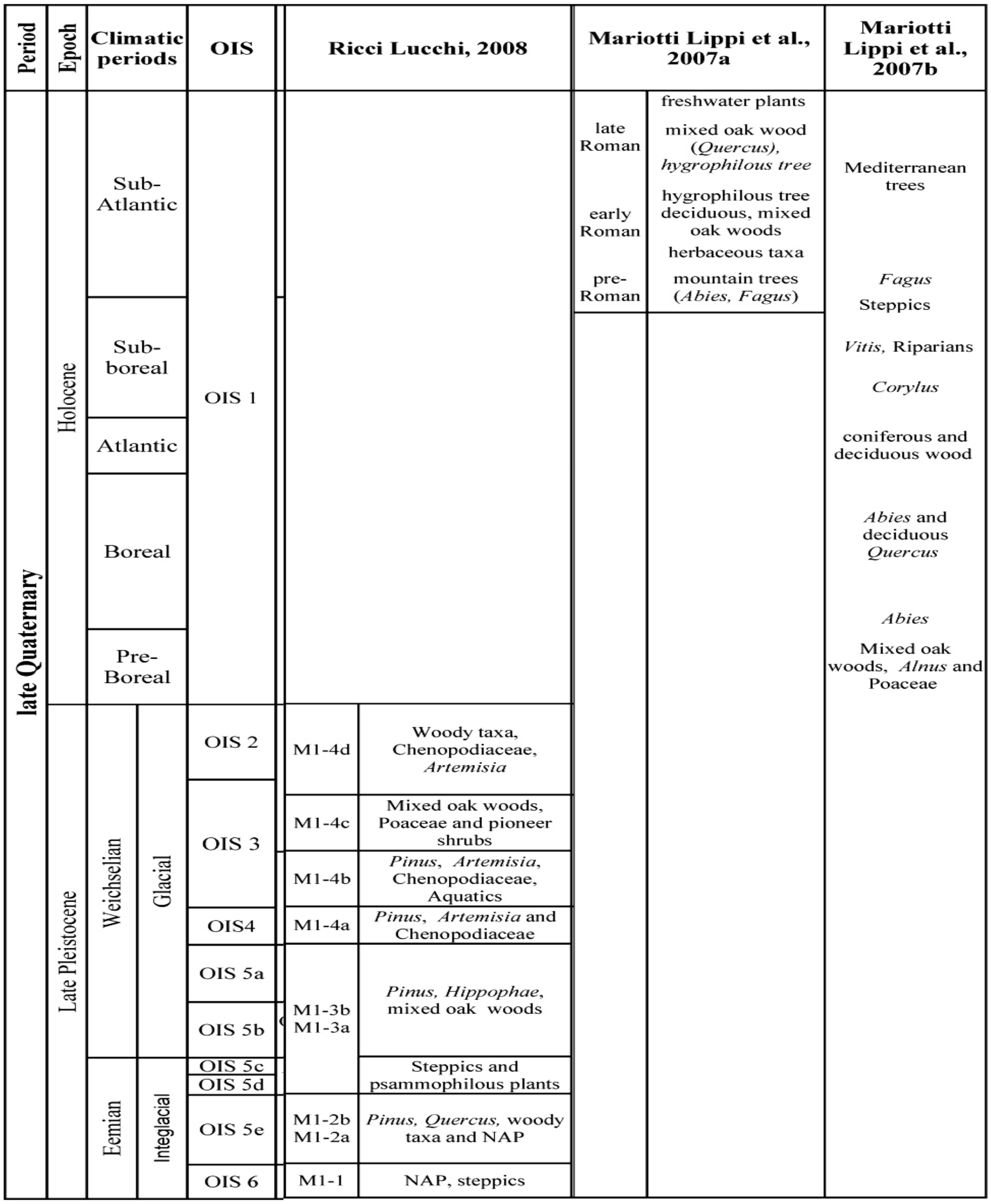
The water table of the forest is extremely limited (Perfetti 2010). The ground water table is in direct contact with the hydrographical network. In periods of heavy rainfall, water rises to the surface of interdunal areas. According to existing records of the San Rossore forest administration, the water table exhibits regular changes with repeating minimum elevations at the end of the dry summer periods (September – October) and maximum levels following the autumn – winter. From a quality point of view, Cherubini and Bertacchi (pers. comm.), argue that the proximity to the sea affects the ground water, particularly as far as dissolved salts content is concerned. When fresh water flowing towards the sea comes into contact with salt water, it tends to float on top (less dense). This phenomenon can occur on the coast line or in tracks of the hydrographical network where the bed level is below sea level (Perfetti, 2010).

2.1.4 The vegetation at San Rossore

2.1.4.1 From Late Pleistocene to the present

Palynology records have been extensively studied at the coastal area of the Arno River, especially at San Rossore. The findings suggest that this part of the Italian peninsula is a place where plant population persisted over multiple glacial-interglacial cycles (Bennett et al., 1991, Pañuellas 1996, Petit et al., 2005, Magri 2007, Mariotti et al., 2007, Lucchi et al., 2008, Rebecai 2011). This can be observed by the past number of long pollen records (Fig. 6) showing continuous presence of thermophilous and mesophilous taxa during the glacial periods (Late Pleistocene, Lucchi 2008) and the postglacial vegetation (Holocene, Mariotti 2007a, b).

During the Late Pleistocene, the records show a transition from shrub Tundra vegetation (*Artemisia*, *Pinus*, *Ephedra*, *Hippophae* and *Juniperus*) to deciduous and evergreen vegetation (*Quercus*, *Betula*, *Tilia* and *Ulmus*) when temperature increased (Ribecai 2011). The survival of *Tilia*, a species that usually rapidly spreads and disappears under interstadial/interglacial climate conditions (Burga, pers. comm.), indicates again that the coastal plain of the Arno River represented an important glacial refuge (Magri 2007).



Period	Epoch	Climatic periods		OIS	Ricci Lucchi, 2008		Mariotti Lippi et al., 2007a		Mariotti Lippi et al., 2007b
late Quaternary	Holocene	Sub-Atlantic		OIS 1			late Roman	freshwater plants mixed oak wood (*Quercus*), *hygrophilous tree*	Mediterranean trees
							early Roman	hygrophilous tree deciduous, mixed oak woods herbaceous taxa	
							pre-Roman	mountain trees (*Abies, Fagus*)	*Fagus*
		Sub-boreal							Steppics *Vitis,* Riparians *Corylus*
		Atlantic							coniferous and deciduous wood
		Boreal							*Abies* and deciduous *Quercus* *Abies*
		Pre-Boreal							Mixed oak woods, *Alnus* and Poaceae
	Late Pleistocene	Weichselian	Glacial	OIS 2	M1-4d	Woody taxa, Chenopodiaceae, *Artemisia*			
				OIS 3	M1-4c	Mixed oak woods, Poaceae and pioneer shrubs			
					M1-4b	*Pinus*, *Artemisia*, Chenopodiaceae, Aquatics			
				OIS4	M1-4a	*Pinus*, *Artemisia* and Chenopodiaceae			
				OIS 5a	M1-3b M1-3a	*Pinus, Hippophae*, mixed oak woods			
				OIS 5b					
		Eemian	Integlacial	OIS 5c OIS 5d		Steppics and psammophilous plants			
				OIS 5e	M1-2b M1-2a	*Pinus, Quercus,* woody taxa and NAP			
				OIS 6	M1-1	NAP, steppics			

Fig. 6: Adaptation of pollen records recorded at the Arno Coastal plain by Ribecai 2011. Periods and climate periods, OIS (Oxigen Isotope Stage) and MI (defines the size of the core used for the drillings).

The work of Mariotti et al. (2007), describes the glacial vegetation (*Quercus*, *Alnus*, *Ulmus*) which persisted during the Holocene and settled among Mediterranean vegetation. This diverse composition has been found until today at San Rossore and has been widely researched (Gellini et al., 1986, Tomei et al., 2004, Lucchi, 2007, Perfetti, 2010, Ribecai 2011).

The following ecosystems are to be found today as observed by Tomei et al., 2004 and Mariotti Lippi et al., 2007):

- At the coastal sandy zone, pioneer halophytes and psammophytes (plants growing on sand) such as *Cakile maritima* and *Ammophila littoralis* are found. In the wetland areas *Phragmites australis*, *Cladium mariscus*, *Typha angustifolia* and *Carex elata* are the most common species.
- Proceeding inland, *Juniperus oxycedrus* subsp. *macrocarp*a, and *Juniperus. communis* appear on the dunes, followed by *Quercus ilex* woods.
- In the interdunal areas the vegetation is formed by *Quercus robur* and *Carpinus betulus*. Where the soil humidity is high and soil texture finer, *Fraxinus angustifolia* grows, or alternatively *Populus alba* together with *Alnus glutinosa* and *Ulmus minor*.
- The riparian vegetation is dominated by *Salix* sp., *Populus* sp., *Alnus* sp. and *Acer* sp. which grow on natural or artificial river bodies (e.g. Arno, Fiume Morto).
- Pine forests became relevant to the landscape in the 1800's and can be found from the coastal sandy zone to inland zones on the dune strips.

2.2 Aims and Hypotheses

At higher latitudes and altitudes where temperate forests grow, tree-ring formation is usually limited by temperature. Around the Mediterranean Sea, at the lowest elevation, tree-ring growth is limited by precipitation. Temperate trees found in the Mediterranean are usually relict from glacial times. *Quercus robur* (oak) and *Fraxinus angustifolia* (Ash) growing in San Rossore forest are examples of refugial species (Bennett et al., 1991). Since they are not autochthones in the Mediterranean, they are expected to be more sensitive to climate and therefore respond to drought events more strongly than well adapted species (like *Pinus pinea*). Studying the response of trees to drought in Mediterranean mesic sites could help us to understand the effects of such events in that region. The San Rossore site (described in chapter 3.1) is highly suitable for this type of studies.

In this thesis, I make use of this site to compare the response of *Quercus robur* and *Fraxinus angustifolia* to drought with that of *Pinus pinea*. The following hypotheses were tested:

H1 *Gleichläufigkeit* is high among species than sites.

Gleichläufigkeit (synchronicity) between species' tree-rings is one of the parameters to show how species are reacting to a limiting factor from one year to the other. In Dendrochronology, the concept of limiting factor states that rates of plant processes are constrained by the primary environmental variable that is most limiting e.g., precipitation in the Mediterranean (Schweingruber 1988). This means that tree growth cannot proceed faster than the amount of precipitation allows, causing the width of the rings (i.e., the volume of wood produced) to be a function of precipitation (Grissino-Mayer, 2004). Tree-rings width is a function of a combination of factors affecting the site where the tree grows. In addition to climate, tree-ring width is also affected by site conditions (e.g., competition, shade, thinning) and regional conditions (e.g., frost events, masting years). If the same species growing in different sites shows good Gleichläufigkeit between the sites, it could mean that the climatic factor is more important than site specific factors.

H2 *Quercus robur* and *Fraxinus angustifolia* respond to drought events more than *Pinus pinea*.

Pinus pinea has been since a long time introduced in the Mediterranean and it has been considered one of the most well adapted species to that region (Gellini et.al 1986, Atollini et al., 1990, Cherubini 1994). *Quercus robur* and *Fraxinus angustifolia* have been growing in the Mediterranean since the last glaciation, when suitable conditions were found southern of the Alps (Bennett et al., 1991, Mariotti et al., 2007, Lucchi 2008). *Pinus* grows on a variety of soils and under a wide range of temperature conditions while *Quercus* and *Fraxinus* have a higher moisture requirement. Since they are not adapted to survive under drought conditions, they would be probably the first species to be affected by it.

H3 *Quercus robur* responds to drought events more than *Fraxinus angustifolia*.

Quercus is the most widespread oak species in Europe (Tessier 1994). Its distribution area extends from Scandinavia to the Mediterranean. Genetic variability could explain the vast dispersal of oak (Haneca et al., 2009) because common Mediterranean oaks like *Quercus ilex* and *Quercus suber* (evergreens) have developed mechanisms (sclerophyllous leaves and bark) to cope with dry summers in the region. These same characteristics are not observed in deciduous oaks like *Quercus robur*, *Quercus canariensis* and *Quercus pubescens*. *Quercus robur* has been considered (Tessier 1994) the least typically Mediterranean of the deciduous oak species. It does not present an average behavior as the other oaks to monthly climatic parameters, despite the diversity of the habitats researched (Tessier 1994). *Quercus* only settles where water is continuously available. *Fraxinus* on the contrary is well distributed in Southern and Eastern Europe growing naturally in riparian and floodplains forests where water table changes are common. Since this study is carried out on a mesic site, water availability changes could favor *Fraxinus*, since it grows in its usual habitat. *Quercus* could therefore respond to drought more than *Fraxinus*, because it is less adapted to such changes.

3 Material and methods

3.1 Target Species

3.1.1 *Quercus robur* L.

Quercus robur L. (English oak) is the most widely distributed of the oak species ranging from the coast of Norway to the Mediterranean. In Italy, it spreads from north to south of the whole peninsula with exception of Sicily and Sardinia (Pignatti 1997).

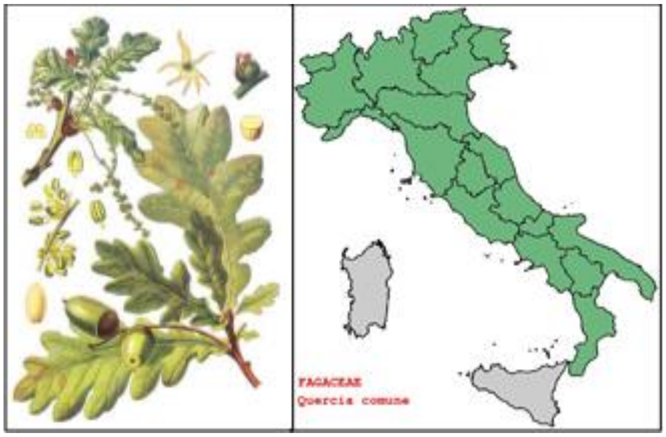


Fig. 7: Quercus robur L. and distribution map in Italy. Source: Flora Italiana online.

Oak has a hygrophilous distribution with progressive concentration to the south in the alluvial plains where the root system resists to periodic submersion, but suffers from the lowering of the water table. It is light demanding and prefers aerated, deep soils, with high pH but tolerates well clay soils as long as they are constantly supplied with water (ARSIA project).

Table 1: Phenology of *Quercus robur* L. Source: ARSIA

Trunk	Root system	Wood	Leaves	Phenology
Average height of about 30 m. Slow growth. Longevity long-lived species (over 300 years).	Long, robust and expanded	Rings are porous.	Yearly deciduous	Seedling: germination underground. Anthesis: contemporary to foliation in April-May, the first flowering usually occurs in 60-70 years. Maturity of the fruit: annually between October and November. Poor resistance to frost.

3.1.2 *Fraxinus angustifolia* Vahl

Fraxinus angustifolia is a deciduous species which is found in most part of Italy, with exception of Sicily and the northern part of the country e.g., South Tirol and Genoa, Torino (Fig. 8). *Fraxinus angustifolia* grows well on moist, rich clays in bottomlands. It does best on rich soils at lower altitudes and will withstand temporary flooding (ARSIA project).

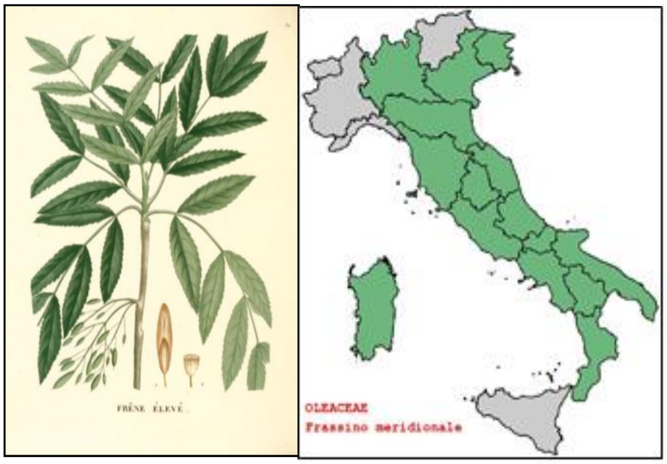


Fig. 8: Fraxinus angustifolia distribution map in Italy. Source: Flora Italiana online.

Fraxinus angustifolia is a light-demanding species with a growing season of 6 to 7 months. For a hardwood, it grows rapidly when young, but its growth slows after 50–60 years. In Mediterranean countries a flowering period is between January/early February and February/early March (Fraxigen project, 2005).

Table 2: Phenology of *Fraxinus angustifolia* Vahl- source ARSIA

Trunk	Root system	Wood	Leaves	Phenology
Average height of about 30 m. Fast growth. Longevity: medium-lived species (maximum 150 years)	Highly developed taproot.	Rings are porous.	Yearly deciduous	Seedling: germination above ground. Anthesis: March-April before foliation. Maturity of the fruit: October. Resistant to frost but sensitive to late frost.

3.1.3 *Pinus pinea* L.

This conifer species has been introduced by early man throughout the Mediterranean and in other regions with Mediterranean climates. It occurs in the Italian peninsula with exceptions of the northern part and some parts in the coast side of the Adriatic Sea (Fig. 9).

Pinus pinea is found in sandy to loamy soils with moisture but well-drained. The soil pH can range from acid to alkaline. It grows well in the shade but prefers little temperature variation.

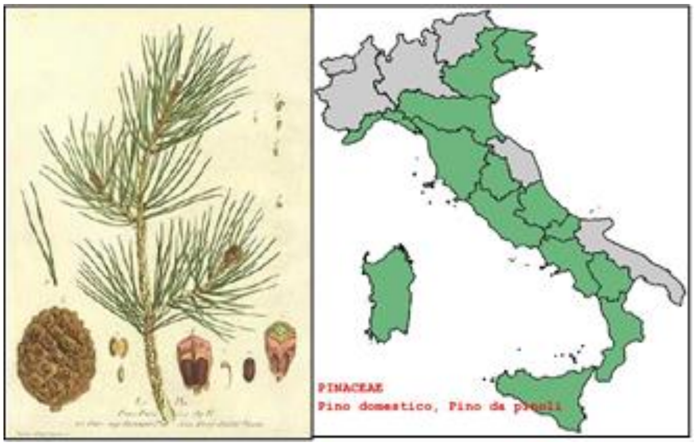


Fig. 9: Pinus pinea L. and distribution map in Italy. Source: Flora Italiana online.

It can tolerate drought and strong winds but it is sensitive to environmental disturbance and will not regenerate easily (Pignatti 1997).

Table 3: Phenology of *Pinus pinea* L. source: ARSIA

Trunk	Root system	Wood	Leaves	Phenology
Average height of about 30 m. Fast growth. Long lived species over 200 years).	Sparse root system, horizontal.	Rings with small cell pours.	Dropped every 2-4 years.	Seedling: germination underground. Anthesis: from May to June. Maturity of the fruit: three years after pollination (the longest maturation period of any pine).The cones open on ripening or up to a year later. Sensitive to disturbances.

3.2 Study Site

The San Rossore forest has three protected sites considered Nature Reserves: Palazzetto (PZ), Lame di Fuori (LF) and Cornacchiaia (CO) which have not received anthropogenic interference (agricultural) for about 40 years, and other sites that have been transformed or used in the past for agriculture as San Piero agrado (SP). The system of dunes and interdunes is present in all three nature reserves. The main difference among sites consists in the proximity to the coast, the soil texture and the association of trees (Fig. 10).

3.2.1 Coast site:

Lame di Fuori (LF) is situated 1.5 km from the coast. It is characterized by a sequence of low dune strips about 1 m.a.s.l. and interdunes at sea level. *Fraxinus* and *Quercus* share the interdunes. The geology is based on alluvial deposits from the flooding of the River Arno; interdunal, retrodunal and filled land fluvial marsh deposits (Holocene): the first are composed of fine sands, from silts and clayey silts, the second are silt deposits with peaty layers. Eolic deposits of dunes from the Holocene are also to be found in this terrain. The interdunes are wet during the rainy season and spring. The orientation of the dunes is Southwest- Northeast.

3.2.2 Inland sites:

Palazzetto (PZ) is situated 4 km from the coast. The dunes are about 20 m high and the interdune is -0,9 m.a.s.l. *Pinus* grows on the high dunes and *Quercus* dominates the interdunes. This site is wet during the whole year. The canopy formed by the trees erases competition from an understory. Palazzetto’s geology is older than the other sites (Pleistocene). It is characterized by medium-fine silty sands and sandy silts. The orientation of the dunes is West-East.

Cornacchiaia (CO) is situated about 2.5 km from the coast and is dominated by *Pinus* growing on low dune strips. On this site the dune- interdune system is not well defined. The other tree species present in the site is mainly *Populus*. Cornacchiaia is 15,2 m.a.s.l and the direction of the dune strips is North-South.

San Piero (SP) is situated about 5 km from the coast. It is known that the old Roman Port was placed at this site. Because of the anthropogenic interference along time, the forest patches in the area are quite young. The vegetation is formed by *Fraxinus*, *Populus*, *Alnus* and a mixture of Mediterranean shrubs. San Piero is formed by alluvial deposits from the River Arno. The soil is distinct by the presence of clay and the proximity to the river keeps the site constantly wet. Dune strips are not noticed as well as in Cornacchiaia.

Table 4: Site, species, number of trees sampled and Lat/Long °C

Site	Species	N.Trees	Lat. (°N)	Long. (°E)
Palazzetto (PZ)	Quercus robur (PZQR)	12	43.74	10.40
	Pinus pinea (PZPP)	7		
Lame di Fuori (LF)	Quercus robur (LFQR)	12	43.69	10.29
	Fraxinus angustifolia (LFFR)	12		
San Piero (SP)	Fraxinus angustifolia (SPFR)	13	43.67	10.34
Cornacchiaia (CO)	Pinus pinea (COPP)	12	43.61	10.34

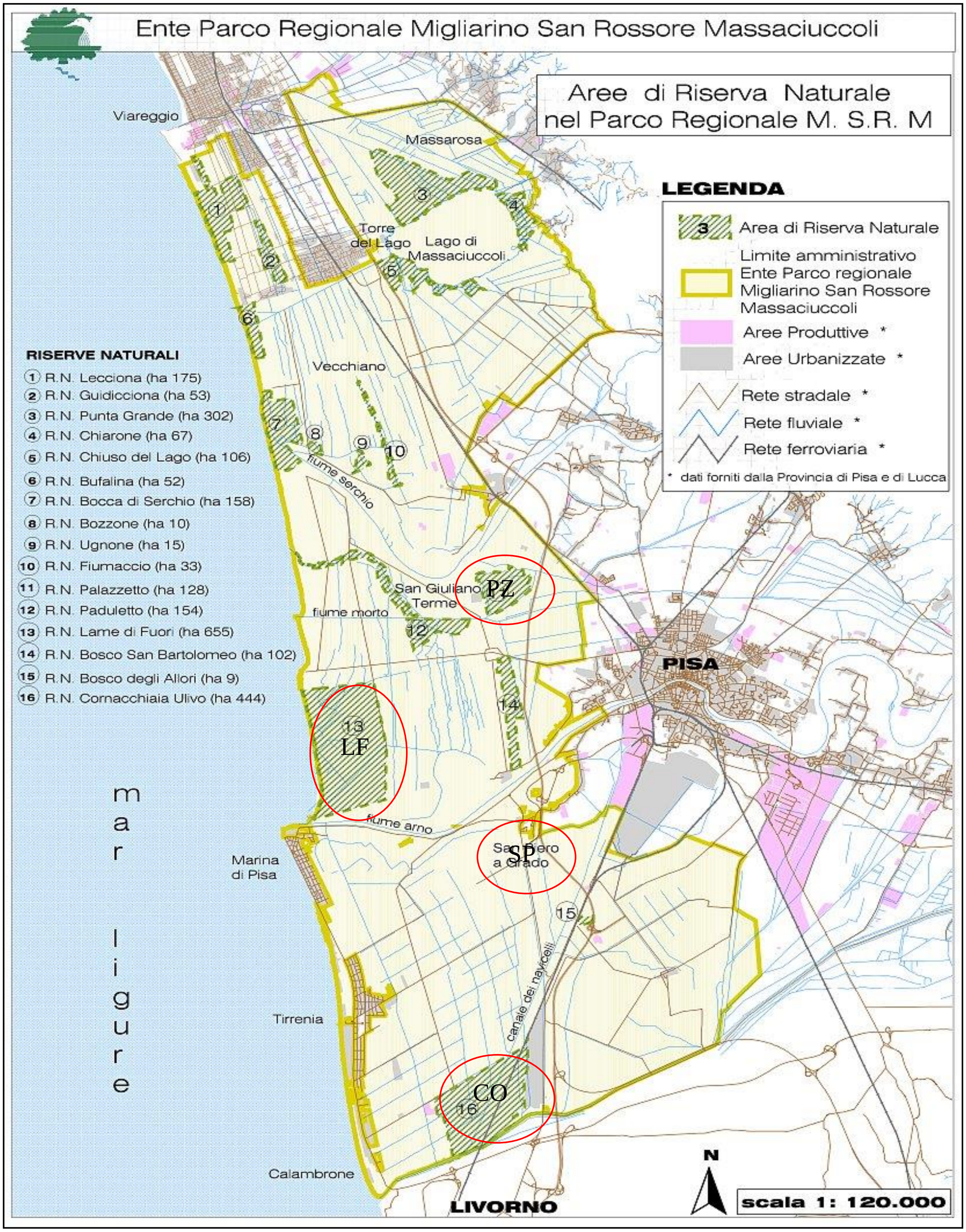


Fig. 10: Sites position within San Rossore forest. PZ (Palazzetto), LF (Lame di Fuori), SP (San Piero), CO (Cornacchiaia). Source: Provincia di Pisa e di Lucca.

3.3 Climatic Data

3.3.1 Available data

San Rossore has a climatic station from which data measured is incomplete in many years and months. Therefore, daily meteorological data were derived from a station of the Italian National Hydrological Service in Pisa (Facoltà di Agraria; 43°43'N and 10°25'E), less than 10 km from San Rossore.

The climatic parameters analyzed were; total monthly precipitation (PREC), mean maximum monthly temperatures (TMAX) and mean minimum monthly temperatures (TMIN). Although the dataset goes back to mid-1800, the complete series were only available from 1922 for PREC, 1944 for TMAX and 1953 for TMIN.

3.3.2 Drought definition for San Rossore

To define what is considered drought at San Rossore, I have calculated the average precipitation of November, December (previous year) and January current year (altogether) from 1922 to 2011 and the standard deviation. I then subtracted the standard deviation from the average. This value indicates the threshold for drought during the winter season. Any value below the threshold would be considered drought. The same procedure was repeated for the months of June, July and August all together in order to define summer drought.

3.4 Sample preparation

3.4.1 Sampling

The trees were sampled from the four sites: Palazzetto inland (approx. 4 km from the coast), San Piero inland (5.0 km from the coast), Lame de Fuori coast side (1.5 km from the beach), Cornacchiaia inland (2.5 km from the coast).

Dominant trees were randomly chosen on each site. Twelve trees were sampled per species. From each tree two cores were extracted using an increment borer at breast height and in an angle of 120° apart from each side of the tree.

3.4.2 Mounting and sanding

Wood samples were put on core mounts and surfaced. After drying they were sanded by file gradients. A belt sander was used and the work was performed firstly with a coarse file of 100-grit to get to a flat surface and then polished further with 150, 220, 280, 320 and 400 grit papers. With a smooth surface the cores' features were enhanced.

3.5 Dendrochronological analyses

3.5.1 Theoretical basis - The Aggregate Tree Growth Model

According to Cook and Briffa (1990), this principle states that any individual tree-growth series can be decomposed into an aggregate of environmental factors, both anthropic and natural, that affected the patterns of the tree growth over time. This is given by a formula (Fig. 11) where:

R_t = the observed ring width series;
A_t= the age size related trend in ring width
C_t = the climatically related environmental signal (e.g. precipitation, temperature)
$D1_t$ = the disturbance pulse caused by a local endogenous disturbance;
$D2_t$ = the disturbance pulse caused by a stand wide exogenous disturbance; and
E_t = the largely unexplained year to year variability not related to the other signals.

$$\boxed{R_t = A_t + C_t + \delta D1_t + \delta D2_t + E_t}$$

Fig. 11: Conceptual Linear Aggregate Model for Tree rings. Source: Cook and Briffa 1990.

The Greek symbol associated with D1t and D2 t is a binary indicator of the presence (=1) or absence (=0) of either class of disturbance at some time t in the ring width.

Grissino-Mayer (2004) also explains that to maximize the desired environmental signal being studied, the other factors should be minimized. To enhance the climate signal from the series of tree ring growth age related trends are minimized using e.g. the software ARSTAN.

3.5.2 Overview of analyses

If I have seen further it is by standing on the shoulders of giants. Isaac Newton

As well said by Isaac Newton, what simplifies the farther progress in dendrochronology is the fact that many scientists have put efforts into developing highly innovative techniques and literature. This detail allowed me to easily access the steps of dendrochronological analysis. The methods previously proposed by Fritts 1989, Schweingruber 1988 and Cherubini et al., 2003 were compiled on the steps below from which a number of dendro software programs have been used:

Table 5: Software programs used on the methods.

Software	Functions	Output
1. TSAP-WIN	Measurement of tree-rings series, calculating correlation among series and crossdating.	Crossdate values. Statistical output from each series (mean, St. deviation, *t*-test, Gleichläufigkeit, CDI)
2. COFECHA	Analyzes crossdating accuracy of all measured series.	Correct correlation among series and statistical values.
3. ARSTAN	Detrending and statistical analysis.	Residual chronology. Calculates Mean sensitivity, standard deviation, Inter correlation of detrended series.
4. DENDROCLIM 2002	Correlating tree-ring residual series with climatic parameters.	Response function and correlation function at statistical significance of 0.05.
5. WEISER – pointer years	Analyses average tree- ring widths and identifies years where positive or negative growth were registered by the same series.	Pointer values which are years where most rings were specially narrow or wide in a specific year.

3.5.3 Dating and cross-dating

A first visual count was done on each one of the cores from bark to pith. This process allows us to mark ring intervals of 10, 50 and 100 years on each sample.

With the help of TSAP-WIN (Rinn 2003), ring widths were measured to the nearest 0.01mm on a LINTAB- measuring table. For every core measured, database was built, by species and site.

The age was compared with the visual count and a first analysis for corrections made from it. Sometimes it is common that cores contain errors from measured, missed or counted rings more than once. In this process the anatomy of the wood may bring additional answers about the tree rings features.

The next step used with TSAP-WIN was cross-dating. It’s the procedure of matching variations in ring width or other ring characteristics among several tree-ring series, allowing identification of the exact year in which each tree-ring was formed (Schweingruber, 1988). Since tree-ring series are never identical, cross-dating involves statistics and the calculation of correlation values to assess the common variability of two tree-ring series. In crossdating, two ring-width series are shifted along each other at 1-year interval. A correlation value is calculated at each position. The correct position for undated series on a dated master chronology is usually characterized by high and statistically significant values.

3.5.4 **Statistical analysis by COFECHA**

The correct dating of measured tree ring series was confirmed by COFECHA (Holmes 1983, Speer 2010), which identifies segments within each ring width series that may have erroneous cross-dating or measurement errors.

COFECHA analyzes crossdating accuracy of all measured series by firstly breaking down each series into shorter segment lengths (32 years this study). It compares each segment with the same calendar segment from the average of all other series by correlation techniques. The analysis then shifts by half the segment length and compares the next 32 year segment, continuing until all series have been tested.

$$r = \frac{\sum_{i=1}^{n}(X_i - \bar{X})(Y_i - \bar{Y})}{\sqrt{\sum_{i=1}^{n}(X_i - \bar{X})^2}\sqrt{\sum_{i=1}^{n}(Y_i - \bar{Y})^2}}$$

Fig. 12 : Correlation coefficients = R-values. Source : Speer 2010.

A segment that falls below the statistical significance threshold is flagged by COFECHA.

It then attempts to place the errant segment in an alternate position via date adjustments which is adjusted manually. The error can be found on a missing or added ring, coming from the measurement or the anatomy of the tree. In case of wrong measurement, the series is adjusted by re-measuring the cores, noting on which year the mistake occurred and re running COFECHA to confirm the correction.

Interseries correlation coefficient (Fig. 12) is the key benchmark of crossdating quality: 0.40. It measures the strength of the common signal in the chronology but it may vary by species and region.

COFECHA also calculates the Mean Sensitivity (Fig. 13) which is the relative change in ring-width from one year to the next and it is positively correlated with interseries correlation. Higher values of interseries correlation and mean sensitivity are often associated with lower autocorrelation.

$$ms_x = \frac{1}{n-1} \sum_{t=1}^{t=n-1} \left| \frac{2(x_{t+1} - x_t)}{x_{t+1} + x_t} \right|$$

Fig. 13: Mean sensitivity formula. Source: Speer 2010.

Although it is not a measure of the chronology's utility for climate reconstruction, it is a good measure of how sensitive the ring widths are, year to year. The effective range is 0.10 to 0.80. For semiarid site trees; 0.35 to 0.50 is common and 0.60 to 0.80 exceptional (Grissino-Mayer 2001).

The series with difficult error detection which affected the overall site chronology were removed. Therefore, some sites only presented 10 series instead of 12.

3.5.5 Statistical analysis by ARSTAN

As mentioned earlier, in order to maximize the climatic signal, noises need to be minimized. To remove high frequency variations from the series Residual Chronologies are calculated using ARSTAN (Cook and Holmes 1999). The program removes non climatic effects and enhances inter annual fluctuations for climate signal in ring width series. Firstly, a negative exponential curve or a linear regression line was fit to the ring series and after a cubic smoothing spline with a 50% frequency cutoff at 32 years was fit to each series allowing low-frequency variability such as age trend to be removed from the series.

Detrending and standardization involved transforming the measured values into dimensionless indices by dividing the raw values (Fig. 14) by the expected values given by the negative exponential and spline functions. Indices were computed as residual from the estimated spline curve.

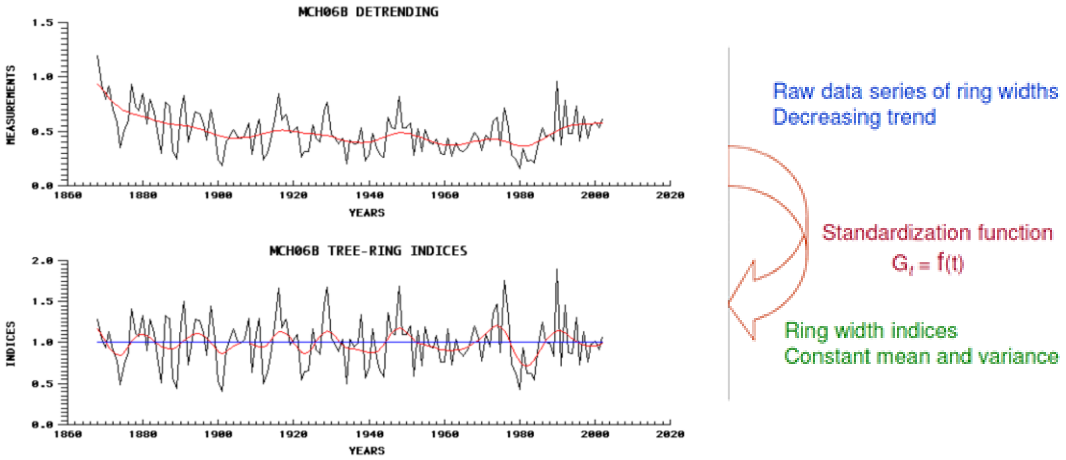


Fig. 14: Transformation of raw tree-ring data into standardized data. Source: Grissino-Mayer 2004 – personal website

For evaluation of quality I applied the dendrochronological statistics on the common period of 1953-2011 for *Fraxinus* and 1922-2011 for *Quercus* and *Pinus*. The period was chosen because the species met the representative number of individuals at this interval.

From the output of Arstan I could use the mean width and standard deviation of the raw ring-width chronology; the first – order autocorrelation of raw chronology, which measures the year-to-year persistence; the mean sensitivity of the residual chronology, which quantifies the relative change in width among consecutive years; the mean correlation among individual series within each site of residual series.

3.5.6 Statistical analysis by DENDROCLIM 2002 program

The complexity of the climate response is so abundant that multivariate methods called response function and correlation function were used to analyze dataset (Fritts 1971). Temperature and precipitation were used in combination with residual output from Arstan statistical program.

From DENDROCLIM 2002 correlation functions and response functions (Fritts 1971) are the outputs. There are two statistical models widely used by dendro researchers.
The term “function” as explained by Biondi et al. (2004), indicates a sequence of coefficients computed between the tree-ring chronology and the monthly climatic variables, which are ordered in time from the previous year growing season to the current-year one. Correlation function (coefficients are univariate estimates of Pearson’s product moment correlation) and response function (coefficients are multivariate estimates from a principal component regression model) were run for all species residual output in combination with climatic data (PREC, TMAX and TMIN). All results are favored by an accurate assessment of statistical significance at the 0.05 level.

I have run the program for response and correlation functions on the residual series output of Arstan for each species with PREC data since 1923, then TMAX since 1944 and at last with TMIN since 1953.

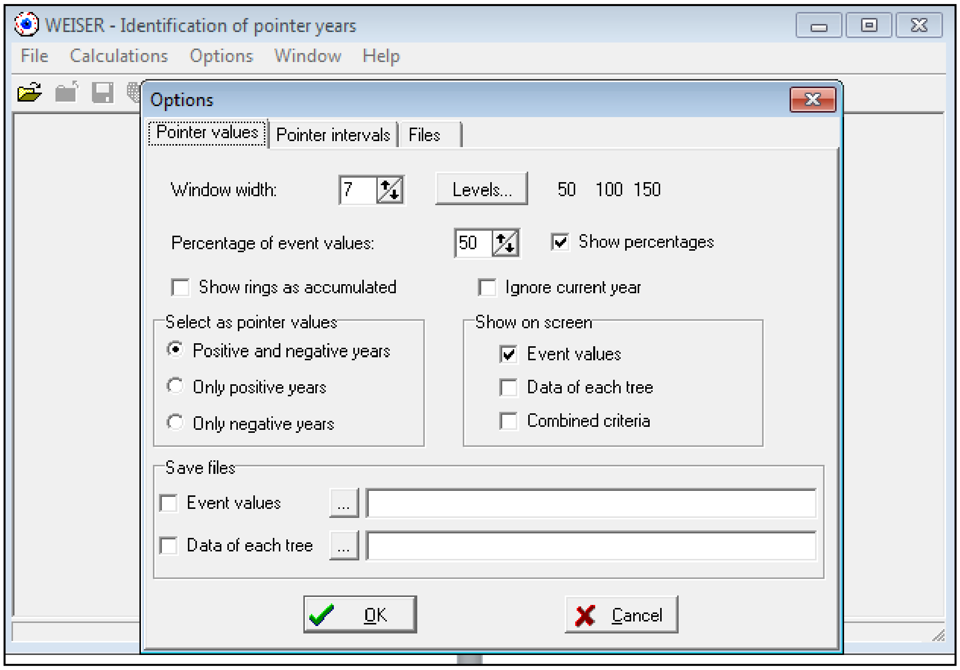
3.5.7 WEISER – Pointer years

To make the connections between climate and radial growth I have used the WEISER Program. It is based on the statistical comparison of long-term growth-ring chronologies with series of monthly meteorological data. Temperature and precipitation for the same period of the series are used.

Using WEISER we can analyze “pointer years”. The term is used to characterize years where events were registered in several tree-ring series. Pointer years refer to a group of trees. According to Schweingruber (1996), they can be measured on the basis of their frequency above a given threshold. The threshold may be based on percentages or statistical significance.

The aim of the threshold depends on the aim of the study. For semiarid trees it can be as high as 50% or more.

In this study I have used average tree-ring width series of each species and the default setting of WEISER which calculates event years with a threshold set at 50% (Fig. 15). The output pointed the years on which all the series of the same species showed 50% of the values with positive or negative growth.



Window width: 7

Percentage of event values: 50

☑ Show percentages

☐ Show rings as accumulated

☐ Ignore current year

Select as pointer values
● Positive and negative years
○ Only positive years
○ Only negative years

Show on screen
☑ Event values
☐ Data of each tree
☐ Combined criteria

Save files
☐ Event values
☐ Data of each tree

Fig. 15: Pointer values default options of WEISER software - Threshold of 50 % of event values- which means a pointer years output are based on 50% or more of the trees with negative or positive abrupt growth.

4 Results

4.1 Samples

At each site, 12-13 trees of each species were cored, totaling 24-26 cores. The longest chronologies were *Quercus* at Lame di Fuori (1796-2011) and *Pinus* at Cornacchiaia (1859-2011).

Fraxinus at Lame di Fuori had the shortest chronology 1939-2011 and was the only species with dated pith. For the other 2 species, the borer probably missed 5-10 cm distance to the pith.

At Palazzetto, there were not enough individual *Pinus pinea* trees on that site and seven trees were cored.

4.2 Site chronologies

The chronologies of each tree species cored were developed. The only difficulties encountered during the measurement of the TRW were imposed by the amount of parenchyma rays on the cores of *Pinus* and the presence of resins that impaired the early to latewood definition. Some cores also presented very narrow rings more than in other series affecting the average TRW and crossdating.

All the species show similar growth patterns at different sites. This is given by the high value of Gleichläufigkeit (synchronicity) which was above 60% (threshold) for all species. That means that at 60% and 2 GSL (**) Pearson’s correlation coefficient is $p<0.01$, at 3 GSL (***) $p< 0.001$. Cross Date Index (CDI) which combines *t*- test and Gleichläufigkeit was above 20 (minimum required is 10) as shown in Table 6.

TRW of *Quercus* and *Fraxinus* correlate quite well between the two different sites; *Quercus* at Lame di Fuori and Palazzetto ($r = 0.55$) and Fraxinus at Lame di Fuori and San Piero ($r = 0.64$), whereas there is no good correlation between the TRW of *Pinus* at Palazzetto and Cornacchiaia (0.06) (Fig. 17)

Table 6: Results of the crossdating within a species between sites *Quercus* (at Palazzetto and Lame di Fuori) *Fraxinus* (at Lame di Fuori and San Piero) and *Pinus* (at Palazzetto and Cornacchiaia): OVL (overlapping years), DateL (left) DateR (right), CDI (Cross date index), TV (*t*-test), GSL (significance level of *Gleichläufigkeit*), GLK *Gleichläufigkeit* between the crossdating of same species in different sites.

	OVL	DateL	DateR	CDI	TV	GSL	Glk %
Quercus	162	1850	2011	41	0.6	***	72
Fraxinus	71	1939	2011	54	6.3	***	74
Pinus	137	1859	2011	20	2.0	**	60

4.2.1 *Quercus robur* (LFQR and PZQR)

The average TRW is around 200 mm for *Quercus* at Lame di Fuori and Palazzetto. No age trend was observed for these series. Gleichläufigkeit is 72 % between the two *Quercus* sites but TRW correlation is not synchronized. TRW series of *Quercus* at Lame di Fuori and Palazzetto are synchronous.

4.2.2 *Fraxinus angustifolia* (LFFR and SPFR)

A clear age trend is present in *Fraxinus* growing at Lame di Fuori and San Piero. Gleichläufigkeit is 74% among the species and TRW correlation is the most significant among all species. From 1958 to 1991, trees at Lame di Fuori have wider TRW than at San Piero. Especially between 1960 and 1970, at Lame di Fuori a disturbance might have caused an abrupt growth release. Since 1980, these species have been growing synchronously.

4.2.3 *Pinus pinea* (COPP and PZPP)

Pinus from Cornacchiaia and Palazzetto showed the lowest TRW synchronicity from all three species. Gleichläufigkeit was low (60%) between these two sites. Their TRW is characterized by different periods of abrupt changes (positive and negative) caused by undetermined events in both sites. At Cornacchiaia it happened during 1933 to 1975. For the same period Palazzetto suffered a decrease on TRW having its lowest growth period in 1959. After 1975 Cornacchiaia went through a gradual decrease in growth while Palazzetto reached a sequence of years with wider tree-rings formed until 1990 (Fig. 16). Since 1999, Cornacchiaia and Palazzetto *Pinus pinea* TRW have been below 300 mm.

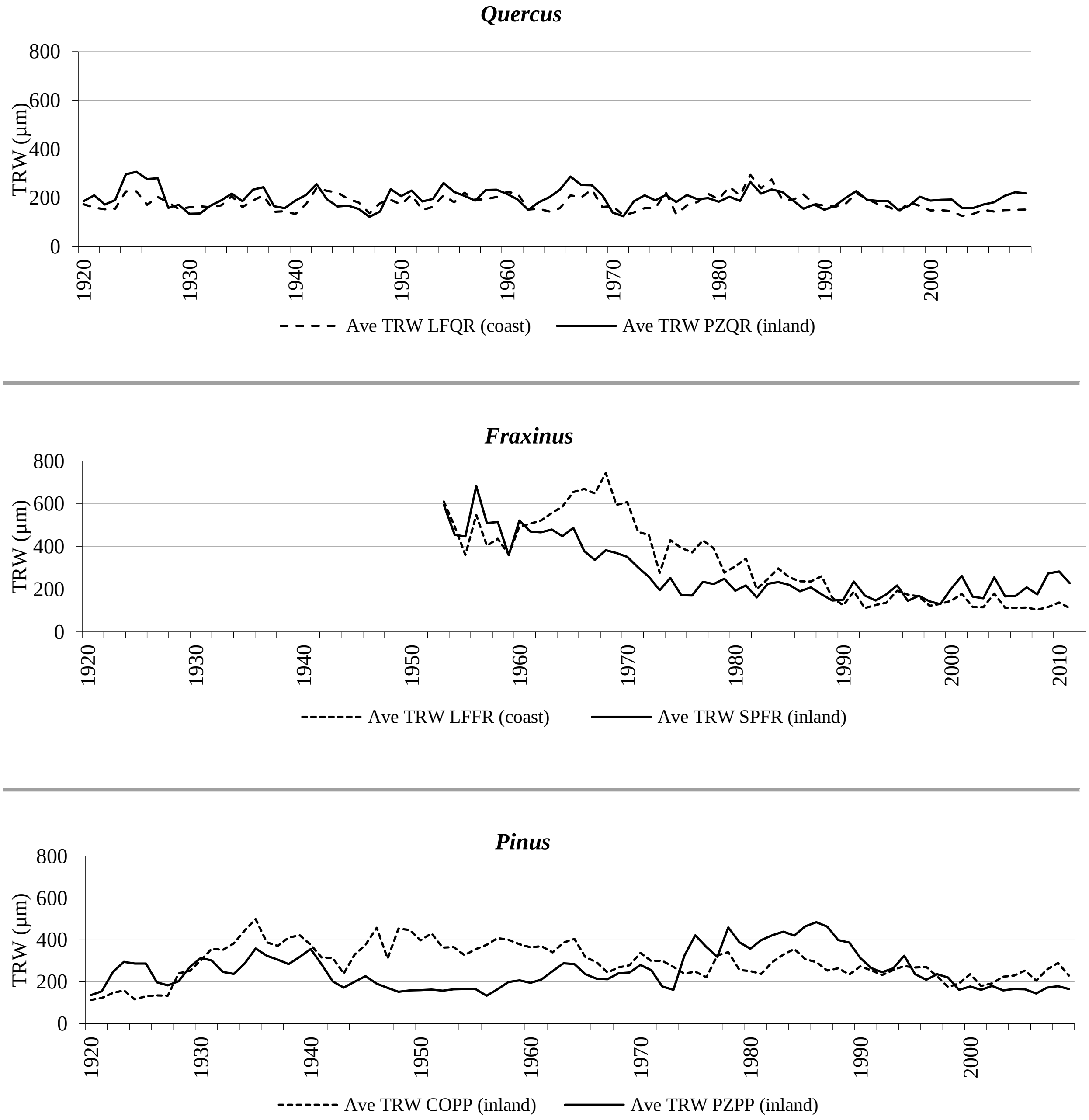


Fig. 16: TRW chronologies within a species between sites: *Quercus* (at Palazzetto and Lame di Fuori) *Fraxinus* (at Lame di Fuori and San Piero) and *Pinus* (at Palazzetto and Cornacchiaia).

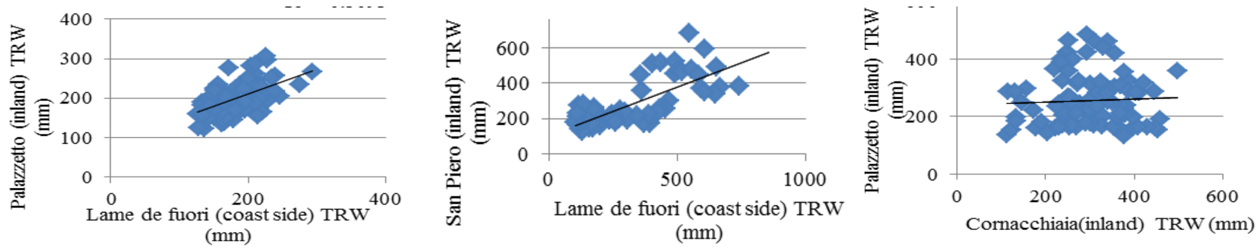


Fig. 17: TRW correlation for *Quercus* (at Palazzetto and Lame di Fuori), Fraxinus (at Lame di Fuori and San Piero) and *Pinus* (at Palazzetto and Cornacchiaia) respectively.

4.3 Sites correlation

In contrast to the correlation between the same species at different sites, the correlation between the different species at the same site is low. In Lame di Fuori although Gleichläufigkeit was good between *Fraxinus* and *Quercus*, TRW wasn't well correlated (Fig. 18). At Palazzetto, *Quercus* and *Pinus* have low Gleichläufigkeit and low correlation between the TRW. An interesting pattern is also found at Palazzetto, where *Quercus* seems to respond to events affecting the site one year after *Pinus* responds to them.

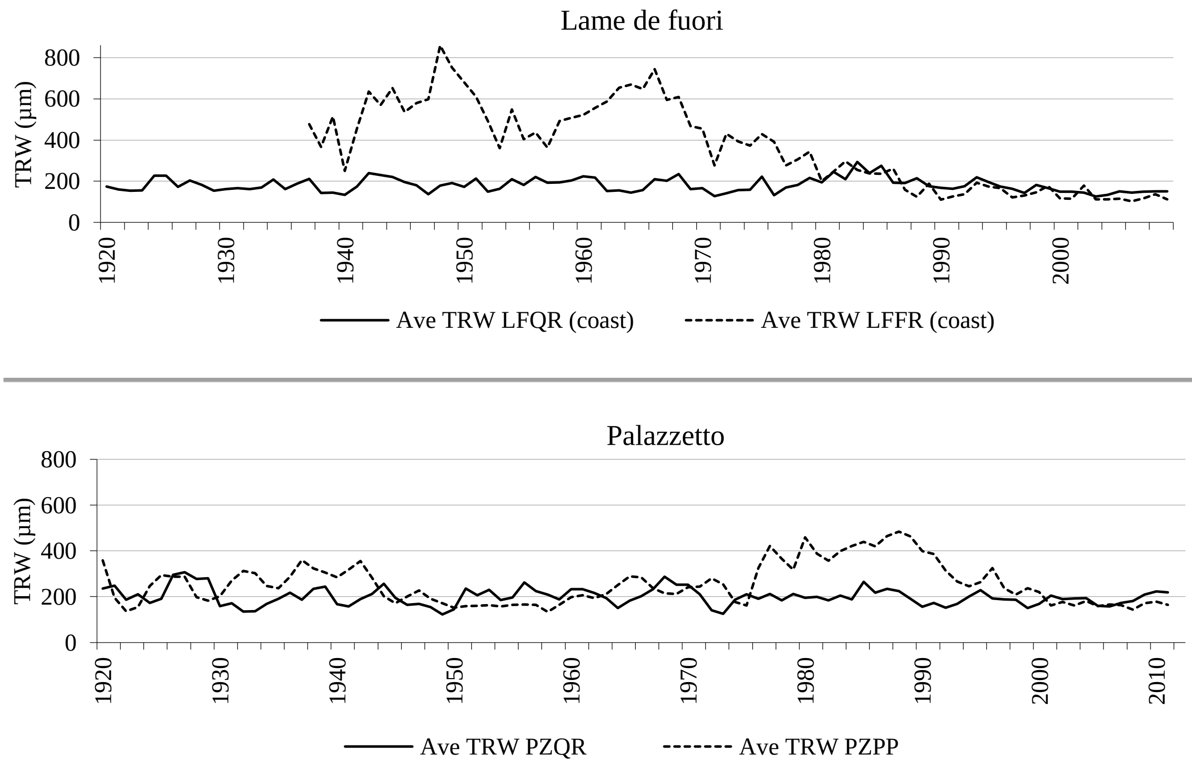


Fig. 18: TRW chronologies within sites: *Quercus* and *Pinus* (at Palazzetto) *Fraxinus* and *Quercus* (at Lame di Fuori).

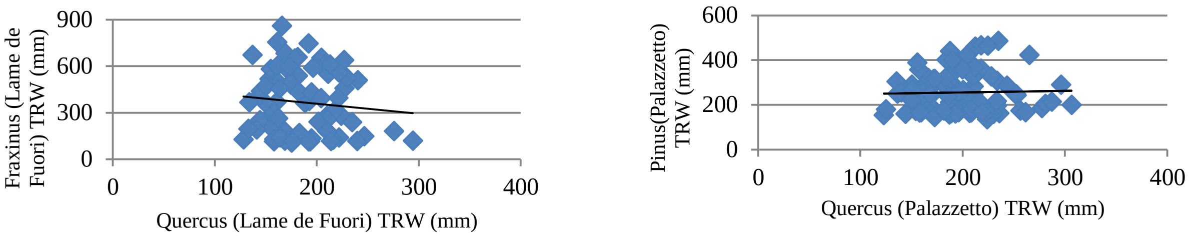


Fig. 19: TRW correlation within sites: *Quercus* and *Pinus* (at Palazzetto) *Fraxinus* and *Quercus* (at Lame di Fuori).

Statistical analysis using COFECHA indicated that cross-dating had some low correlation series within the master chronology. We removed from LFQR and PZQR one tree-core; LFFR and COPP two tree-cores because they were outliers compared to their master chronology.

Mean sensitivity which is the sum of the total signal (age trend, climatic variations, exogenous and endogenous factors) was the highest (0.313) for *Fraxinus* at San Piero and lowest for *Pinus* at Cornacchiaia (0.208, Table 7). Series inter-correlation is a measure of how well all cores from one same species correlate among themselves. It is the highest for *Fraxinus* (0.490) at San Piero and lowest for *Pinus* (0.393) at Palazzetto. Mean TRW ranged around 0.3 µm with exception of PZPP (0.102 µm, Table 8)

Table 7: COFECHA output- Number of trees used, average mean sensitivity which shows how much high frequency variance (noise) is present and series inter-correlation (correlation among all cores of one species).

Site	Species	Time span	Number of trees	Average mean sensitivity	Series inter-correlation
Palazzetto (PZ)	*Quercus robur* (PZQR)	1922-2011	11	0.240	0.471
	Pinus pinea (PZPP)	1922-2011	7	0.264	0.393
Lame di Fuori (LF)	*Quercus robur* (LFQR)	1922-2011	11	0.258	0.441
	Fraxinus angustifolia (LFFR)	1953-2011	10	0.285	0.459
San Piero (SP)	*Fraxinus angustifolia* (SPFR)	1953-2011	13	0.313	0.490
Cornacchiaia (CO)	*Pinus pinea* (COPP)	1922-2011	10	0.208	0.454

Statistical analysis with Arstan confirmed that even after detrending (removing age trend, and other noise in the data), *Fraxinus* at San Piero and Lame di Fuori had the highest mean sensitivity value while Palazzetto and Cornacchiaia had the lowest (Table 8). Mean sensitivity is one of the most important parameters to express the reactivity of trees to climatic events. A high mean sensitivity from a detrended chronology means good response to climatic signal (Table 8).

Table 8: Statistical values drawn from Arstan output: Mean sensitivity which shows the amount of climatic signal on each chronology (after removal of the noise), Standard deviation, Mean TRW.

Site	Species	Time span	Number of trees	Mean sensitivity	Standard deviation	Mean TRW - µm
Palazzetto (PZ)	*Quercus robur* (PZQR)	1922-2011	11	0.16	0.14	0.282
	Pinus pinea (PZPP)	1922-2011	7	0.15	0.14	0.102
Lame di Fuori (LF)	*Quercus robur* (LFQR)	1922-2011	11	0.18	0.17	0.350
	Fraxinus angustifolia (LFFR)	1953-2011	10	0.20	0.16	0.266
San Piero (SP)	*Fraxinus angustifolia* (SPFR)	1953-2011	13	0.25	0.20	0.329
Cornacchiaia (CO)	*Pinus pinea* (COPP)	1922-2011	10	0.14	0.21	0.297

4.4 Climatic data

Precipitation is the most important factor in San Rossore. Precipitation NDJ (November, December previous year plus January of the current year) and JJA (June, July and August of the current year), was the longest set of climatic data and therefore the best comparisons for TRW series. Temperature was relatively low in the mid 1950's, end of 1970's and end of 1980's, however a slight increase in temperature (especially during the summer) occurred after 1990 (Fig. 20). Precipitation fluctuates more than temperature within the periods observed. Precipitation JJA is marked by specific years when summer precipitation was expressively low (around 40 mm) in 1931, 1938, 1945, 1962, 1964, 1985 and 1993. The period 1972 – 1977, precipitation NDJ was at its lowest. The year with the most abundant precipitation NDJ and JJA occurred in 1963. From 1922 – 2011, precipitation NDJ was below 185 mm (which is the drought threshold for winter precipitation, cf. chapter 3.3.2) during 16 years (18%).

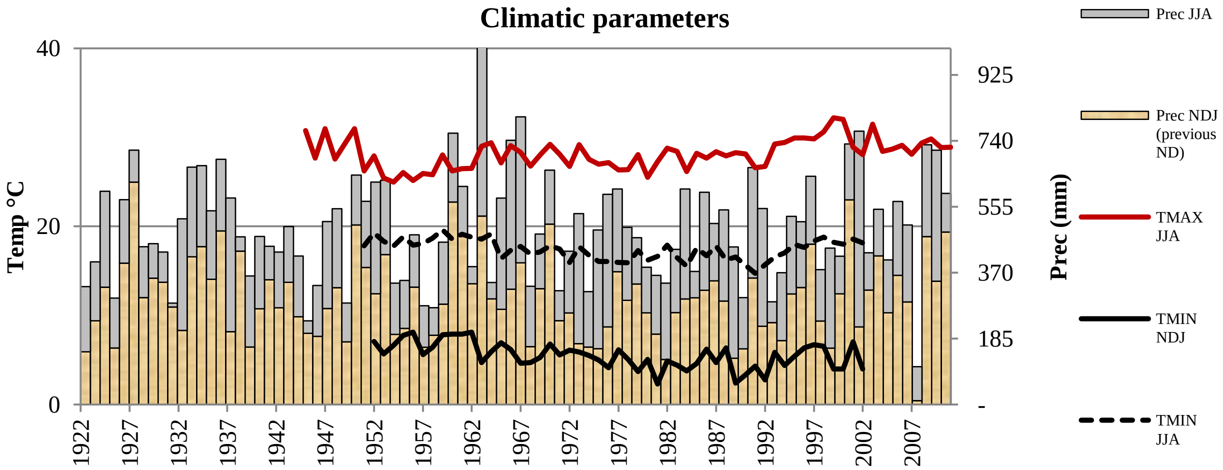


Fig. 20: Climatic parameters from Pisa Facoltà di Agraria: this Fig. illustrates the fluctuations of PREC JJA and NDJ (ND previous year) since 1922, TMAX JJA since 1944, TMIN NDJ and JJA from 1951-2003.

4.5 Climate-tree-ring growth relationship

The analysis between climate parameters and tree-ring growth was only possible to be determined for TMAX and determined for TMAX and PREC which have the longest series. Ring widths of all species are positively correlated with positively correlated with previous year November - December precipitation and January-February of the current year. February of the current year. Temperature only correlated positively and negatively with few species (

species (

Fig. 21: Correlation and response function between TRW and climatic parameters (TMAX and PREC). Sig P corr and Sig RRFF are significant correlation function and response function respectively (p<0.05). The figure displays the good correlation of all species with pointer years.

18).

4.5.1 Quercus robur

Neither of the two *Quercus* sites showed a significant correlation with temperature. TRW is positively correlated at Palazzetto during December of previous year and January the current year. At Lame di Fuori correlation to precipitation is significant (p<0.05) during November and December of the previous year. A significant negative correlation is observed in LFQR during September of the current year. Better correlations are found at Lame di Fuori than Palazzetto (Fig. 16).

4.5.2 Fraxinus angustifolia

Fraxinus TRW at San Piero correlates negatively with June temperature, but not at Lame di Fuori. At the latter site, the temperatures during October and November of the previous year as well as the April temperature of the current year correlate positively with TRW.

In both sites *Fraxinus* shows a positive correlation with precipitation but at Lame di Fuori it is particularly stronger for the month of November and May.

4.5.3 Pinus pinea

At Cornacchiaia TRW correlates negatively with September temperature. *Pinus* TRW is positively correlated with precipitation in January-February and August of the current year.

At Palazzetto no significant correlation was observed with temperature. At this site TRW correlates positively with December precipitation of the previous year and January precipitation of the current year.

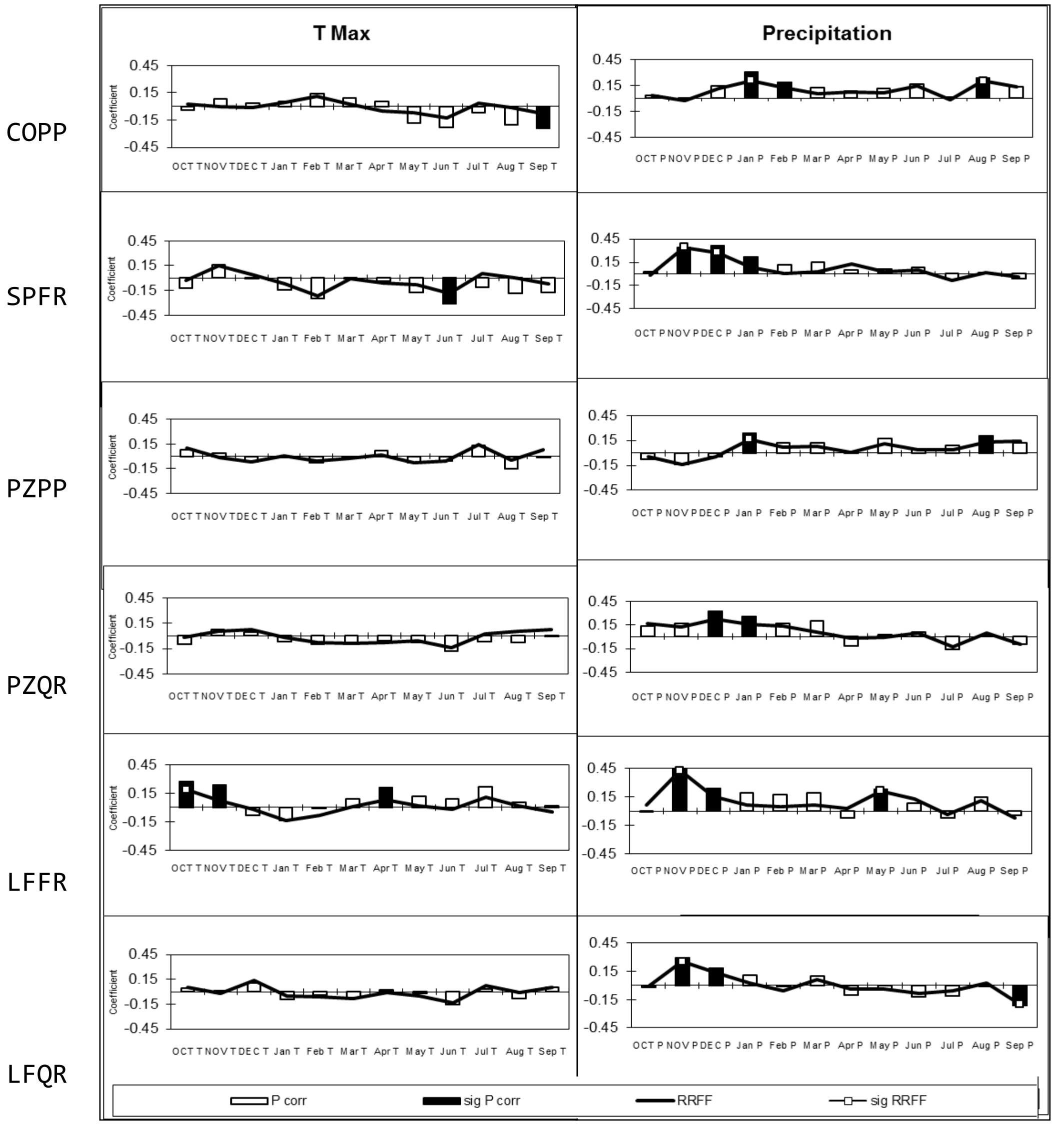


Fig. 21: Correlation and response function between TRW and climatic parameters (TMAX and PREC). Sig P corr and Sig RRFF are significant correlation function and response function respectively (p<0.05). The figure displays the good correlation of all species with pointer years.

LFFR

Only few pointer years were detected. Analysis resulted in the identification of eight negative pointer years for COPP, six for PZQR, five for PZPP, three for LFQR, LFFR and SPFR each (Table 9).

A detection of drier/wetter winters and summers was facilitated by contrasting pointer years with NDJ (November, December previous year with January current year) and precipitation JJA (June, July, August current year) (Fig. 22).

LFQR

In 1968 however, the low NDJ precipitation was also coincident with positive pointer years, formed by PZQR and LFFR. Summer precipitation JJA was below 90mm (drought threshold in summer) during 11 years (12%).During this 89-year period, 23 years were negative pointer years for at least one species and one site. Six of these 23 years (26 %) precipitation NDJ (< 185 mm) and two of these 23 years (9%) precipitation JJA (< 90mm) matched the formation of negative pointer year.

4.5.4 Quercus robur

Observing the PYs of *Quercus* at both sites, it appears that these trees haven't been suffering because of drought. *Quercus* TRW does not correlate with temperature but it correlates positively with winter precipitation.

At Palazzetto, negative pointer years are apparently formed 2-3 years after summer precipitation was low and previous year winter precipitation was below 185 mm (e.g., 1949, 1972 and 1999). However, some years are not well correlated with the low precipitation such as 1964 when (previous winter precipitation) had been above average for 6 years and the summer precipitation on that exactly year is the second highest on the century. Positive PY's for PZQR were formed after about 3 consecutive years when summer and winter precipitation were higher than the average (e.g. 1968, 1985). *Quercus* at this site correlates positively with previous year winter precipitation.

At Lame di Fuori, only few negative and positive PYs were observed. In 1929 and 1949, the negative PYs were formed after winter precipitation being below 185 mm and after 3 years when summer precipitation was below 20 mm. In 1977 only LFQR built a positive pointer year after a gradual increase of precipitation during the previous eight years (Fig. 22). Nevertheless, a negative PY resulted in 1978 although winter and summer precipitations were within average. Positive PY's were more abundant at Lame di Fuori than Palazzetto usually in years of high previous winter precipitation.

4.5.5 Fraxinus angustifolia

LFFR and SPFR had three negative PY's starting from 1959 which happens to be the only match at both sites. At Lame di Fuori this 1959 and the 1972 negative PY were formed 3 years after an abrupt decline of winter precipitation in 1956 and a gradual decline from 1969 to 1972. As for San Piero, *Fraxinus* also seems to respond negatively to these decreases in precipitation in previous winter precipitation forming PY's in 1982 and 1999.

There were more positive pointer years observed in SPFR than at LFFR. With the exception of 1968, *Fraxinus* apparently built wider rings after high previous winter precipitation.

4.5.6 Pinus pinea

COPP negative PYs were formed on the period of 1922 to 1983. PZPP has its five PYs during 1922-1958 from which only one (1922) is coincident with COPP. It is possible that in the case of this species, summer precipitation might have affected TRW much more than in the other species. Observing years 1929, 1958, 1969, 2000 and 2001 where some negative PY were formed only the current summer precipitation was very low. The previous 2-3 years before the formation of these negative PYs were considerably wet. *Pinus* positive PYs are more present in Cornacchiaia than Palazzetto however; they are not obviously related to the amount of precipitation. Apparently growth release happened more than once on each of the sites.

Table 9: Negative Pointer Years (PY): by year when they occurred and by site- species. In grey are years when 2 species formed negative PYs, in Red when 2 species formed opposite PYs (for one species is positive and the other negative) and Green when 2 species had positive PY's. Prec NDJ below 185 mm and 90mm for Prec JJA is considered drought and "X" are years coinciding with PY's.

Negative Pointer years	COPP (inland)	PZPP (inland)	PZQR (inland)	LFQR (coast)	LFFR (coast)	SPFR (inland)	Prec. NDJ < 185 mm	Prec. JJA < 90 mm
1922	-1	-1					**X**	
1923		-1						
1924			-1					
1925	+1			-1			**X**	
1929		-1						
1944		-1	+1					
1945	-1							**X**
1949			-1	-1			**X**	
1956	-1							
1958		-1						
1959					-1	-1		
1964			-1					**X**
1968			+1		+1		**X**	
1969	-1							
1972		+1	-1					
1973			-1		-1		**X**	
1976	-1							
1978				-1				
1982						-1	**X**	
1983	-1							
1992					-1			
1999			-1			-1	**X**	
2000	-1							
2001	-1					+1		
SUM	**-8**	**-5**	**-6**	**-3**	**-3**	**-3**	**7**	**2**

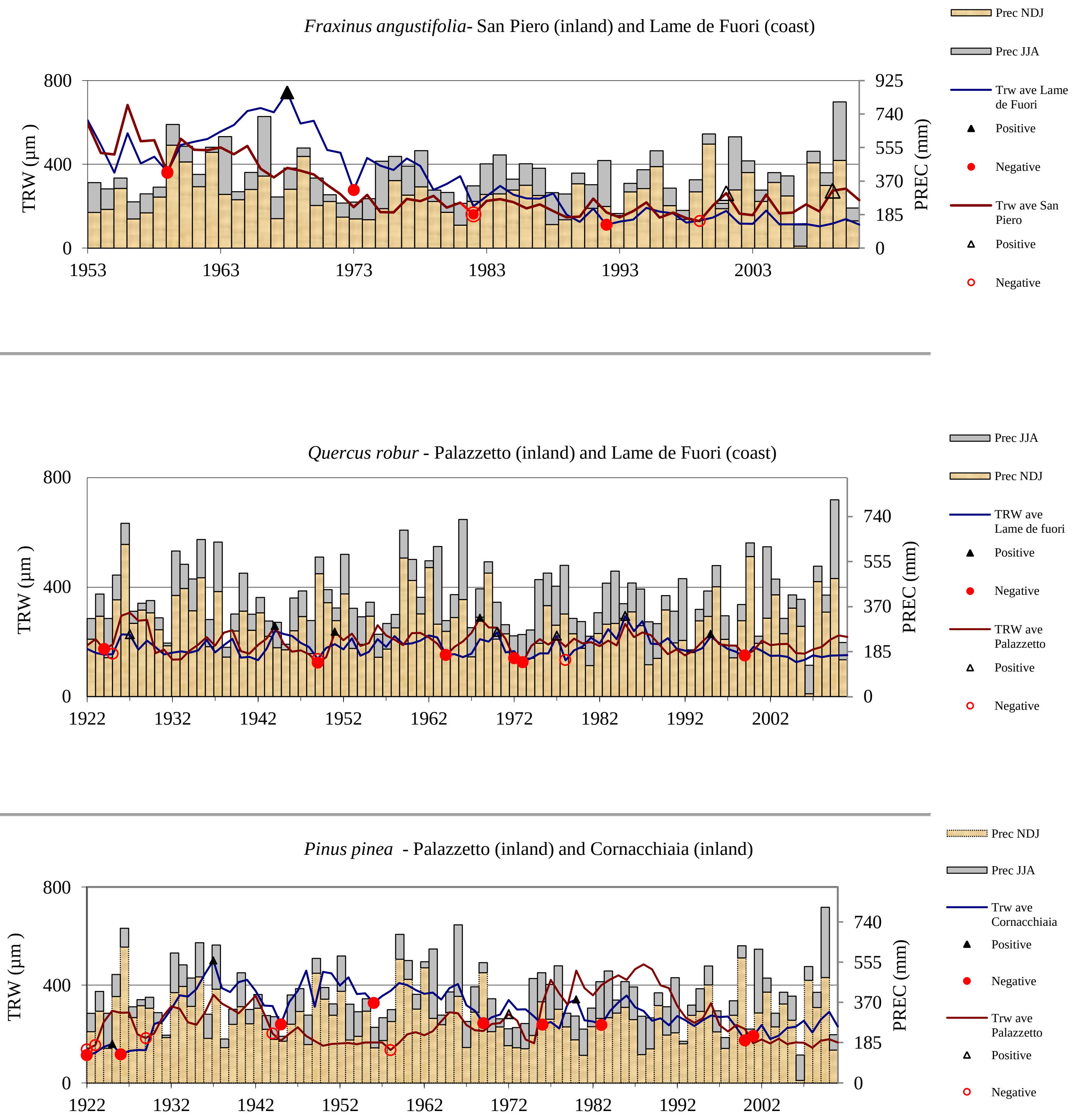


Fig. 22: Pointer years per species *Fraxinus* at Lame di Fuori and San Piero, *Quercus* at Palazzetto and Lame di Fuori and *Pinus* at Cornacchiaia and Palazzetto. The dotted peaks are years when 50% (or more) of the trees at each site answered positively or negatively to an event.

5 Discussion

5.1 Methods

Dendroecological analysis relies on statistical methods to remove noise and unwanted trends from the data. Although the methods used in this study are well established, debates still exist on data standardization (Briffa and Melvim 2011). During the analytical procedure, subjective decisions (e.g., choice of the detrending method) might influence the result. In order to minimize this effect I consistently used the 32 year (as commonly used in COFECHA) spline for all three species. I also tested several types of regressions and I noticed no difference e.g, between a negative exponential and a 32 years smoothing spline processed by the ARSTAN program. Consequently, the results can be considered to be robust.

Another problem I have encountered in the methods was the difficulty to find a long series of climatic data in Pisa. The trees cored were older than 200 years, whereas the digitally available climatic data was only available since 1921 (90 years).

5.2 Climate/ tree-ring width relationship

5.2.1 Species specific response to drought

In general the results have shown that for all the studied species, the tree-ring width is not well correlated with temperature, but mostly significantly correlated with precipitation. The mean sensitivity of the residual chronologies which indicates if a species is sensitive to climate variability is considerably low for all species (MS between 0.15-0.25). Maximum temperature (of any month) does not correlate well with ring width, which could indicate that these species are not suffering because of high temperatures. Negative and positive extreme growth periods were not synchronous (Fig. 22). Never more than two species showed extreme growth peaks in a same year, which suggests that environmental factors other than climate are affecting the formation of tree -rings at these sites.

Drought at our sites was defined by when precipitation is lower than 185mm during the winter and 90 mm during the summer. It was possible to connect a couple of years with negative growth and find a reason for their occurrence:

Fraxinus is the species that we hypothesized would have shown significant correlation with climatic events, because it shows higher mean sensitivity than all other species (Table 8) and fairly good correlations with precipitation and temperature on specific months (Fig. 21). Nevertheless, *Fraxinus* has formed very few pointer years, of which 1982 and 1999 are correlated with dry winter, i.e., precipitation at San Piero, and 1973 at Lame di Fuori. Their occurrence could be explained by this species vulnerability to fall-winter precipitation, especially in November. While my data suggests that dry winter affects growth of *Fraxinus*, other studies have shown the effect of low winter temperature. At extreme low temperatures, especially seed production is often low or even non-existent in *Fraxinus*, so that frost is likely to be a major factor limiting the species' distribution (Piotto and Piccini 2000).

In the case of *Pinus*, which presented more negative pointer years than all other species, a significant positive correlation with August precipitation possibly means that ring width could have been affected when summer conditions were very dry. A negative pointer year was observed at Cornacchiaia in 1945 when summer precipitation was below 90mm, and all other negative pointer years were formed apparently when current summer precipitation was lower than previous summer precipitation. Only one negative pointer year (1922) was related to winter precipitation. Some studies have already shown that the weather conditions during the late spring-summer period are the drivers of *Pinus pinea* radial growth (Cherubini 1994, Piraino et al., 2013). Because they grow on sandy dunes at San Rossore, with a low water storage potential they would suffer more from water deficiency than the other species growing in wetter and more clayish soils. Therefore, negative pointer years in *Pinus* are related rather to dry summers than dry winters.

Quercus and *Fraxinus* cell division is reduced by water stress, as it delimits the amount of nutrients available on the formation of new cells (Fonti et al., 2011). This does not seem to be a problem given the mean tree-ring width of each species chronology and the low number of negative pointer years they built.

There was no indication that *Quercus robur* was limited by temperature. No correlation has been observed with tree-ring growth in any month's temperature at Lame di Fuori or Palazzetto. Monthly average temperature and precipitation values can explain 5–72% of the variation in annual tree-ring-widths of living oak trees (Pilcher and Gray 1982, Rozas 2001) However, south of the Alps and in the Mediterranean, above average May, June and July temperatures have been observed to have a negative effect on ring-widths (Santini et al., 1994, Čufar et al., 2008). *Quercus* typically responds to drought during the winter. I could relate the negative tree-ring growth with below 185mm winter precipitation during 1949 for *Quercus* at Lame di Fuori and Palazzetto, 1973 and 1999 for *Quercus* at Palazzetto. On all three years, *Quercus* at Palazzetto was the most consistent species to respond to dry winters, which means that if precipitation decreases in winter *Quercus* at Palazzetto could be the first one to sense it.

Two examples illustrate that the species have very different requirements for seasonal distribution of precipitation: at Palazzetto, in 1944 when summer precipitation was scarce but winter precipitation was abundant, *Quercus* formed a wider ring and *Pinus* a narrower ring. The opposite happened in 1973 and the species answered in the opposite way, highlighting that these species relationship to specific seasonal precipitation needs for growth.

5.2.2 Complex interactions between climate and other factors

I have attempted to match similar ecological features on all four sites at San Rossore (Cornacchiaia, Palazzetto, Lame di Fuori and San Piero) where the trees were sampled. There is some evidence that climatic factors control the growth of these species. The results of this study, suggest that precipitation is the limiting factor for *Fraxinus*, *Quercus* and *Pinus*, because the growth of all species correlated significantly with precipitation. *Fraxinus* was the only species significantly correlated with temperature (TMAX). In a previous work conducted on long-term changes in precipitation (annual precipitation amount and intensity) and temperature in Pisa (Moonen et al., 2001), the results showed that after 1940 there was a decline in winter and spring precipitation intensity. I have used the dataset from the meteorological station of Pisa Facoltà di Agraria, which provides complete digital data since 1922 and is located approximately 10km from our sites at San Rossore. Moonen et al. (2001), from the same station, used a longer dataset, available as printed version but not digitally. Despite this decrease in precipitation during the winter and spring after 1940 (as measured by Moonen et al., 2001), there was no obvious effect on the growth of either species (contrary to what could have been expected).

What we found to be agreeing with the Moonen et al. (2001) results is that temperature after 1990's increased slightly and that 1997 was indeed the warmest year of that decade. Nevertheless, trees at San Rossore didn't react instantaneously to increasing temperatures. First, an increase in evapotranspiration would be expected, affecting trees and soil evaporation influencing the water table. In our results, the trees only formed narrow rings when, additionally to warm temperatures, precipitation was very low during the winter. This type of response can indicate that an additional factor may help these trees when temperatures are high. Woody plants growing in the Mediterranean exhibit a wide phenological diversity which cannot be explained just on the basis of climatic constraints (Castro-Diez et al., 2003). Local ecological influences could modify the species response to drought apart from the climatic factors. Such observations are identified because there was a lack of similarities among chronologies when limiting conditions were present. Climate is considered one of the main but not the only driver of tree growth because of site and species specific properties (e.g. Frank and Esper, 2005).

In an earlier study, Santini et al. (1994), have reached this same conclusion and suggested that the abundance of water at the sites would create enough evaporation to form the effect of a cloudy day which would block radiation and lower the effect of high temperatures on the sites. In this study, I could not make the same observations because no related data was collected. However, I have detected the presence of a closed canopy and a high leaf area cover that could be the reason for which the trees in San Rossore do not react as much to warmer temperatures. The sites Lame de Fuori and Palazzetto did not present an understory at those sites where the trees were sampled, which might confirm the lack of sun light available for this forest layer. If solar radiation is blocked by the canopy, cooler temperatures are registered inside the forest. The soil will not be as much affected by evaporation and therefore present good water availability. Shade could facilitate growth by cooler temperatures in summer and warmer temperatures in winter (Gómez- Aparicio et al., 2006) modifying the climatic characteristics of the sites and consequently the behavior of each present species (Aussenac 2000).

Shade tolerance could be the microclimatic driver in addition to precipitation (macroclimatic driver) because trees did not form narrow rings when temperatures were very high but they formed narrow rings when dry conditions occurred. Therefore, shade could induce different responses among species because of the interaction between low light and limited water availability (Valladares and Niinemets, 2008).

5.3 Hypothesis evaluation

5.3.1 H1 *Gleichläufigkeit* is high among species than sites.

This hypothesis is supported by the results of crossdating. Trees of the same species generally showed synchronicity of the pattern of their tree-ring chronologies, although this synchronicity was better for *Fraxinus* and *Quercus* than for *Pinus*. However, synchronicity of the chronologies does not necessarily result in a good correlation of tree-ring widths between the same species growing at two different sites. In the case of *Pinus pinea*, no correlation is observed between the TRW at Cornacchiaia and those at Palazzetto.

One of the basic principles in dendrochronology is that similar growth conditions result in similar ring-width patterns (Schweingruber, 1988). Therefore, stress caused by extreme climatic events could be expected to affect all trees at the same region in a similar way.

Given the wide extension of the forest at San Rossore and the distance between sites, it is presumable that these old forest stands might be responding to a regional climatic factor when it comes to same species, because the distance amid sites conserved the growth synchronicity between them. However, different species growing in the same site are reacting to different local factors, because they don’t show any good synchronicity.

5.3.2 H2 *Quercus robur* and *Fraxinus angustifolia* respond to drought events more than *Pinus pinea*.

The results suggest that each of the three species shows a specific response to drought and that a direct comparison is therefore not possible.

This hypothesis cannot be confirmed only based on a regional climatic response. Each one of these species' response to drought is apparently related to a specific factor. This means that different species are not limited by the same climatic parameters at the same time.

What could be observed is that *Pinus* tree-ring growth is constrained by water stress during summer previous to growth, as suggested by the negative relationship with September temperature, and to the positive relationship with precipitation at the end of the summer. The same observations have been reported in an earlier study by Andreu et al. (2007). *Pinus* developed narrow rings more often than the other species when water conditions were limiting during the summer. This suggests that it responds to drought during the summer more than *Quercus* and *Fraxinus*. In this region, drought occurs regularly in the summer; during this period precipitation is usually insufficient which causes depletion of soil moisture (Kozlowski, 2002). A reduction in plant growth is then expected normally during the summer.

In contrast to *Pinus*, *Quercus* and *Fraxinus* are less affected by summer drought and rather affected by winter drought (as discussed on chapter 5.2.1).

The response of *Quercus* and *Fraxinus* to strong droughts is therefore different from that of *Pinus* but not necessarily stronger. The results suggest that the season, when drought occurs, is an important factor controlling the growth reaction of the species. While *Pinus* reacts more strongly to summer drought than *Quercus* and *Fraxinus*, the opposite is expected in the case of dry winters. In this case, *Quercus* and *Fraxinus* will probably answer more than *Pinus*.

5.3.3 H3 *Quercus robur* responds to drought events more than *Fraxinus angustifolia*

Our results support the hypothesis that *Quercus* forms narrow rings more often during dry winters than *Fraxinus*. During the period 1922-2011 this was the case for *Quercus* at Palazzetto (1949, 1973, and 1999) and Lame di Fuori (1925 and 1949), and for *Fraxinus* narrow rings caused by dry winters were only observed three times, at Lame di Fuori (1973) and San Piero

(1982 and 1999). *Quercus robur* is considered the less adapted species of the genus to the Mediterranean (Tessier et al., 1994). Nevertheless, *Quercus* is the dominant species in the interdunes at San Rossore and dominant trees are more likely to respond to climatological pulses over larger areas, whereas suppressed or shaded trees are more influenced by the local forest dynamics (Haneca et al. 2009). This is one of the reasons why *Quercus* might be answering to droughts during the winter more than *Fraxinus,* but it is not the only one. There might be also a close relationship to how much the water table oscillations influence positively or negatively tree-ring width.

5.3.3.1 Species reactions to water excess

One of the most important physical aspects at San Rossore is that the water table is quite high throughout the year. Especially at Lame di Fuori and Palazzetto, water on the ground surface is present even during the summer. Under these conditions, the presence of water in the soil could be positive but also negative. Tree response to water submersion may vary according to the survival strategy of the species (Glenz et al. 2006), since in this case they would develop one strategy for drought times and another for water logging.

Observing the behavior of *Fraxinus* at San Rossore, leafing comes early in the year, around February. It could be a strategy to compete with other species and guarantee growth (Piotto and Piccini 2000). At Lame di Fuori, *Fraxinus* grows together with *Quercus* so that this earlier start of the vegetative period would enable it to profit from the water. When *Quercus* leaves come later by mid-March to early May, this species then competes for water and light with *Fraxinus*. Mérian and Lebourgeois (2011) have shown that tree size and canopy position significantly mediate climate/ tree growth relationships of deciduous trees. At Lame di Fuori, *Fraxinus* and *Quercus* ring-width patterns after 1982 are similar. Probably, *Fraxinus* reached the canopy at this year, and started competing (Fig. 15). Because once the crowns have reached the canopy and became exposed, evapotranspiration rises, resulting in increasing demand of soil water. On well drained soils as it is at Lame di Fuori, this effect is often observed and described by foresters who say “when soil water is high at the end of the of January, after *Fraxinus* start leafing in early February the water level lowers”. Because *Fraxinus* is light and water demanding, without this strategy it would struggle to survive on this site.

The strategy of *Quercus robur* could be related with its root system which is able to reach deeper layers in the soil (Haneca et al., 2009). *Quercus* is among the few broadleaved species that are able to develop roots also in water saturated deeper layers (e.g., Kutschera and Lichtnegger 2002, Glenz et al., 2006), which should be the case at Palazzetto where *Quercus* grow in clayish soils. *Fraxinus angustifolia* is considered the most water logged tolerant species of the genus (Glenz et al. 2006, Fraxigen 2005). One of the main ways in which plants adapt to water logging involves the capacity of aerial tissues to absorb O_2, transport through the stems for the purpose of increasing absorption of macronutrients by the roots and the oxidizing of toxic compounds in wet soils into no-toxic compounds (Kozlowski 1984, Armstrong et al., 1994). Morphological adaptations, such as hypertrophied lenticels, aerenchyma tissues and adventitious roots, increase the uptake of O_2 by aerial tissues and transport into the root system (Glenz et al. 2006). *Fraxinu*s sp. develops all three coping mechanisms (Frye and Grosse 1992, Siebel et al., 1998, Polomski and Kuhn 1998) while *Quercus robur* only develops adventious roots and lenticels (Siebel et al., 1998, Schmull and Thomas 2000). This can suggest that *Fraxinus* tolerates waterlogging well, because of these three coping mechanisms, whereas *Quercus* has a slight disadvantage. However, water logging did not cause injuries in either species, probably indicating that they both benefit from it. *Quercus* sensitivity to dry winters is higher and could be explained because it is used to be in contact with the water table, since it has deep roots, and suffers if water is not available, whereas *Fraxinus* makes use of the water strategically adjusting to seasonality.

6 Conclusions

In this study, I have assessed the tree-ring response to drought of *Fraxinus angustifolia* and *Quercus robur* in comparison to *Pinus pinea* at San Rossore, Pisa. It was the first dendrochronological investigation studying two relict species on San Rossore's dune-interdune systems.

Comparisons between the species enhanced our understanding about their relationship between their ecological behaviors at San Rossore, especially their individual responses to drought.

Overall, there was no strong drought signal because the sites are relatively wet. I defined drought by calculating a threshold for low precipitation during the summer and winter. During these two seasons, tree growth showed the best correlation with precipitation.

From the hypothesis two of the three could be confirmed;

H1- there is synchronicity among same species growing in the different sites, confirming that they are all limited by the same macroclimatic factor, in this case precipitation. The different synchronicity within different species at a same site shows that microclimate has also an influence on their growth and that species have individual adaptive strategies to profit from the water availability.

H2- *Fraxinus* and *Quercus* do not have more narrow rings than *Pinus* when it comes to low summer precipitation. Because *Pinus* has less access to water since it grows on dunes. Therefore, low summer precipitation is the main limiting factor for *Pinus*. *Fraxinus* and *Quercus* build narrow rings when winter precipitation is scarce. The hypothesis can therefore not be answered generally, but seasonal differences have to be taken into account.

H3- *Quercus* does respond to drought more than *Fraxinus*. When conditions were limiting during the previous winter, *Quercus* has grown more narrow rings than *Fraxinus*. Possibly because *Quercus* is the dominant species in the interdunes and it is highly dependent on continuous water availability while *Fraxinus* has developed strategies to adapt to the oscillations of the water table.

The main conclusion of this study is that with increasing temperatures and decreasing precipitation in the future, *Quercus robur* will probably be the first to suffer from drought events since it is highly dependent on continuous water availability. After continuous droughts, the growth of *Quercus* could decline, allowing species such as *Fraxinus angustifolia* to spread and possibly dominate mesic areas, because of its good adaptation mechanisms observed at San Rossore.

For future research of tree-rings and climate at San Rossore I would suggest that: firstly documented climatic data prior to 1900’s, which have never been digitalized, would be considered. Moonen et al. (2001) used this data but I did not have access to it. Secondly instead of tree cores, I would suggest that if possible cross-sections would be sampled, because the trees at San Rossore are old and present much additional information on ecological aspects that could not be seen in tree cores. Thirdly it would be interesting to add pedological and hydrological investigation associated with the sites studied, to understand how soil properties are affecting and being effected by the forest stand itself (shade, soil temperature, texture, water table fluctuation etc.). Forth, by including other species in this type of studies (e.g., *Alnus glutinosa*, *Populus alba* and *Carpinus betulus*), a more general understanding of the role of *Quercus robur* and *Fraxinus angustifolia* for this landscape could be achieved.

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11 List of abbreviations

ARSTAN – Autoregressive Standardization

COFECHA – Cross dating (in Spanish)

COPP – Site Cornacchiaia species *Pinus pinea*

JJA – June, July and August of current year

LFFR – Site Lame di Fuori species *Fraxinus angustifolia*

LFQR – Site Lame di Fuori species *Quercus robur*

NDJ –November and December of the previous year and January of current year.

PREC – Mean monthly precipitation

PY – Pointer years

PZPP – Site Palazzetto species *Pinus pinea*

PZQR – Site Palazzetto species *Quercus robur*

SPFR – Site San Piero species *Fraxinus angustifolia*

Sig P CORR - Significant correlation function ($p<0.05$)

Sig RRFF - Response function ($p<0.05$)

TMAX- Mean monthly maximum temperature

TMIN – Mean monthly minimum temperature

TRW – Tree Ring Width

Personal declaration

I hereby declare that the submitted thesis is the result of my own, independent, work. All external sources are explicitly acknowledged in the thesis.