

Monitoring 2 REINFFORCE Network Arboretums

Health Status, Growth and Phenology

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“It is not the strongest of the species that survives, not the most intelligent that survives.

It is the one that is the most adaptable to change.”

Charles Darwin, 1809 - 1882

Resumo

As alterações climáticas estão a modificar a composição vegetal e animal nos ecossistemas florestais. Compreender como as árvores e os insetos herbívoros estão a lidar com estas alterações é crucial para planear as plantações florestais de modo sustentável. Usando dois arboretos pertencente à rede REINFFORCE, visámos analisar como diferentes espécies e proveniências são afetadas por danos causados por agentes abióticos e bióticos. Visámos ainda analisar a influência da fenologia na herbivoria, tendo por modelo duas espécies de carvalhos, uma autóctone e uma exótica, e as suas proveniências.

No geral houve diferenças significativas na sobrevivência das diferentes espécies. Todavia, a sobrevivência não diferiu entre coníferas e folhosas ($p = 0.659$). Dentro de alguns géneros, como seja *Pinus*, verificou-se maior sobrevivência das espécies nativas comparativamente às exóticas. Tal relação não se verificou nos carvalhos.

A descoloração foi o maior tipo de dano verificado nalgumas espécies, em particular na alfarrobeira, *Ceratonia siliqua*, sugerindo a presença de factores abióticos de stress, possivelmente ligados ao clima e solo. Os valores de desfolha foram mais elevados em dois géneros de folhosas, *Quercus* e *Fagus*. O arboreto de Lisboa teve mais danos e maior número de pragas do que o de Sintra, o que poderá estar relacionado com as diferenças climáticas dos dois locais e o estado vegetativo e fisiológico das árvores, apresentando estas maior vigor no arboreto de Sintra.

No geral, os danos por herbívoros foram menores em espécies exóticas do que em nativas. Na avaliação de herbivoria nas duas espécies de Carvalho estudadas ao longo da época de primavera, mostrou-se que a espécie nativa *Quercus robur* sofreu três vezes mais dano do que a espécie exótica *Quercus rubra*. Adicionalmente, mostrou-se que o dano causado por herbívoros esteve diretamente relacionado com a fenologia das proveniências, provando-se a influência deste fator.

Palavras-chave: Exótica, nativa; Sobrevivência; Herbívoro; Fenologia; REINFFORCE.

Abstract

Climate change is affecting plant and animal composition in forest ecosystems. Understanding how trees and insects are coping with climate change is most relevant to develop sustainable forest plantations, in the future. Using two REINFFORCE network arboreturns, we aimed to analyse how different species and provenances are affected by abiotic and biotic damaging. Additionally, we also aimed to assess phenology influence on herbivory, having as model two Oak species, a native and an exotic, as well as their provenances.

In general, there were significant differences in survivorship from different species. However, it did not differ between coniferous and broadleaved species ($p = 0.659$). It differed at genus level, such as *Pinus*, where we observed a higher survivorship on natives when compared to exotics. This relation was not verified in oak species.

Discolouration was the main damage type verified in some species, in particular on Carob tree, *Ceratonia siliqua*, which suggests the presence of abiotic stressors, possibly linked to climate conditions and soil. Defoliation values were higher in two broadleaved genera, *Quercus* and *Fagus*. Lisbon arboretum had higher damaging and pests than Sintra arboretum, which might be related with the climate differences on both sites and also, the physiological and vegetative status of trees, which presented higher stamina in Sintra.

Overall, herbivore damaging was lesser in exotic species than native ones. In herbivory assessment from the two studied Oak species, along Spring season, it was shown that the native species *Quercus robur* suffered three times more damage, than the exotic species *Quercus rubra*. Additionally, we showed that herbivore damaging was directly related to phenology development from provenances, thus proving this factor influence.

Keywords: Exotic vs native; Survival; Herbivory; Phenology; REINFFORCE.

Resumo Alargado

As alterações climáticas criam alterações profundas nos ecossistemas. Nesta sequência, torna-se importante conhecer o modo como as árvores e outros seres vivos estão a lidar com as mesmas, para que possamos contribuir para a conservação de espécies florestais. É importante ainda, saber como adaptar os povoamentos florestais de produção a novos cenários mantendo-os sustentáveis.

Neste trabalho foram usados dois arboretos pertencentes à rede da infraestrutura REINFFORCE. Esta infraestrutura foi criada nas regiões atlânticas europeias, entre as latitudes 37° e 57°N, com o objectivo de ajudar na compreensão da capacidade que as diferentes espécies florestais têm, para lidar com o clima futuro. Cada um dos arboretos continha 33 espécies, cada uma das quais com um mínimo de 3 proveniências, de forma a existir qualidade e quantidade genética suficiente. Em Portugal foram instalados 6 arboretos, 3 no arquipélago dos Açores, na ilha de S. Miguel, e 3 em Portugal continental, em Sintra, Lisboa e Vila Real. Neste estudo, acompanhou-se o estado de saúde das árvores nos arboretos de Lisboa e Sintra com o objetivo de estudar diferenças de adaptação entre espécies e proveniências a estes dois locais com características climáticas distintas. Pretendeu-se ainda comparar a fenologia durante a época de primavera de proveniências da espécie nativa *Q. robur* e da espécie exótica *Q. rubra* e relacionar com o consumo por insetos herbívoros.

Na colheita dos dados foram utilizados os protocolos estabelecidos pela infraestrutura REINFFORCE. Foram usados os protocolos para o acompanhamento da fenologia, assim como o protocolo de avaliação de danos por agentes bióticos e abióticos. Para a realização dos trabalhos relativos à fenologia e sua relação com a herbívora foram realizadas visitas de campo semanais para observação destes parâmetros.

Na realização da colheita de dados fenológicos, acompanhou-se o desenvolvimento completo das folhas, desde o gomo até à folha adulta, sem ocorrência de crescimento dos ramos. Para este estudo foram selecionados os ramos mais próximos com os pontos cardeais, em todas as árvores alvo. Nas medições, utilizámos apenas 8 estágios a escala BBCH adaptada à infraestrutura, dos quais se selecionou o estágio de completo desabrochar das folhas como diferenciador de árvores temporãs e tardias.

Nos dados de herbivoria, utilizaram-se três tipos de dano e suas classes: i) mortalidade na copa com 5 subclasses, ii) descoloração na copa com 6 subclasses, e iii) desfolhação na copa com 6 subclasses. No total analisaram-se 20 tipos diferentes de dano, causados por agentes bióticos e abióticos, registando-se a presença ou ausência dos mesmos.

Relativamente à análise sanitária do arboreto, constatou-se no geral que houve diferenças significativas entre espécies e géneros, mas que a sobrevivência não variou significativamente no global entre espécies exóticas e nativas. No entanto, quando a sua variação foi verificada a nível de género, foram observadas diferenças entre espécies exóticas e nativas nalguns géneros, em particular no género *Pinus*. A justificação para estas diferenças pode dever-se à não adaptação de espécies exóticas às condições climáticas dos locais de estudos, já verificado em estudos anteriores (e.g.: Correia *et al.* (2018)). Apesar destes resultados, verificou-se que espécies exóticas provenientes de zonas climáticas semelhantes poderão estar melhor adaptadas a sobreviverem na região do estudo, obtendo-se sobrevivências iguais, ou superiores, às nativas. Justifica-se assim os valores de sobrevivência elevados observados para espécies exóticas como *Quercus shumardii* e *Cupressus sempervirens*, entre outros.

Os danos por herbivoria (desfolhação) no arboreto de Lisboa foram mais evidentes nas espécies de folhosas do que em resinosas. Este resultado pode dever-se à existência de uma maior quantidade de pragas especializadas no consumo de folhosas, ou nos diferentes mecanismos de defesa inerentes a cada grupo de espécies. No geral, as exóticas mostraram menores valores de desfolhação que as espécies arbóreas nativas, facto que poderá ser justificado pela não coevolução das espécies exóticas com as comunidades de espécies herbívoras nativas.

No geral, sobressaiu ainda o facto de existir uma maior percentagem de descoloração do que desfolhação, o que nos leva a crer que existe uma maior quantidade de agentes de stress abiótico nos locais em estudo, em particular no arboreto da Tapada da Ajuda. É possível que a qualidade do solo e/ou condições climáticas do local não sejam as mais adequadas ao desenvolvimento de algumas das espécies. Todavia, observou-se uma elevada e heterogénea variação dos dados entre géneros e espécies. Os géneros *Quercus* e *Fagus* obtiveram maiores valores de desfolhação comparativamente aos restantes. Por outro lado, os maiores valores de descoloração foram registados na espécie *Ceratonia siliqua*.

Dos vinte tipos de dano avaliados, apenas foi verificada a presença de dezassete. Verificou-se ainda que os tipos de dano foram altamente específicos consoante o género florestal em questão. Por exemplo, o cancro e seca apenas foram verificadas no género *Pinus*, enquanto os desfolhadores (tritadores, esqueletizadores e mineiras), assim como os fungos causadores de ferrugem e míldio ocorreram maioritariamente no género *Quercus*.

Das três espécies mais utilizadas na indústria florestal portuguesa, a espécie nativa de pinheiro bravo, *P. pinaster* registou os menores valores de desfolhação e descoloração. Por

outro lado, *Quercus suber* obteve maior desfolhação e *Eucalyptus globulus* obteve maior descoloração.

No estudo da fenologia e herbivoria, realizado ao longo da época de primavera, verificámos que a herbivoria evoluiu de acordo com a fenologia. As diferenças entre espécies e proveniências foram significativas. A espécie nativa *Quercus robur* desenvolveu as folhas mais cedo do que a espécie exótica. Também a nível de proveniência, observou-se que três subgrupos diferiram significativamente entre si relativamente ao número de dias julianos necessários para alcançarem o estágio de desenvolvimento correspondente às folhas expandidas. Verificou-se ainda que o consumo por herbívoros foi tanto maior quanto menor o tempo necessário ao início do desenvolvimento das folhas. Justifica-se este resultado pela existência de sincronia entre a atividade dos insetos e a presença de folhas e uma maior janela temporal passível de ser usada pelas pragas provando assim a influência crucial da fenologia na herbivoria. Neste último ponto verificámos assim a existência de uma relação de proporcionalidade direta entre a fenologia e a herbivoria, com uma correlação quase perfeita ($R^2 = 0.9667$).

Concluindo, as alterações climáticas são o desafio do século com o qual todos teremos de lidar. Infraestruturas como o REINFFORCE são um contributo para que possamos melhorar a nossa compreensão sobre como diferentes espécies florestais e proveniências poderão lidar com as mesmas. Com este estudo, comprovámos diferenças significativas entre espécies e proveniências, relativamente a danos causados por agentes bióticos e abióticos. Observámos também uma influência do clima e dos solos com expressão em maiores danos observados no arboreto de Lisboa comparativamente a Sintra. A influência da fenologia sobre a herbivoria, bem como a existência de diferenças nos danos causados a espécies nativas e exóticas, foram evidenciadas neste estudo. Esperamos que este estudo contribua para uma melhor compreensão do estado atual de ecossistemas florestais e que, aliado a outros estudos, possa resultar numa ferramenta de aconselhamento para a criação de futuros povoamentos florestais. No futuro pensamos que seria benéfico haver mais estudos deste teor, comparando sobrevivência de espécies de árvores exóticas e nativas em diferentes climas, bem como estudos a avaliarem a variabilidade da incidência de pragas florestais em diferentes espécies arbóreas. Estes estudos permitirão compreender como se irão desenrolar os eventos relacionados com a sanidade florestal em face às alterações globais.

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1. Introduction

1.1. The relevance of forests

Globally, forests cover around 4 billion hectares (ha) of land, or 30% of the Earth's land surface, where its majority is covered with primary forests (36%) or modified (53%) natural forests. Still, the primary forest area has been slowly decreasing by an average of 6 million ha annually since 1990s (Kirilenko & Sedjo, 2007). Land use activities have transformed a large proportion of the planet's land surface, resulting from the use of natural resources for human needs, often at the expense of environmental conditions (Foley *et al.*, 2005). The loss of large areas of natural forest was one of the main consequences. The loss of these forest areas tends to be higher in low-income countries, largely in the tropics, and mostly due to the direct influence of human activities. On the contrary, higher-income countries have reversed their earlier forest losses and are recently experiencing forest expansion (Kirilenko & Sedjo, 2007).

According to data published by Eurostat, in the "Agriculture, forestry and fishery statistical book", the European Union (EU28) accounts for approximately 5% of the world's forested area, with almost 182 million ha. Aside from it, European forests are among the most intensively managed forests in the world, counting with only 0.4% of non-managed protected forests (Jactel *et al.*, 2009). Also, according to data published by Food and Agriculture Organization of the United Nations (FAO), the forest area in Europe is currently increasing by approximately 0.4% per year, which corresponds to 760 000ha per year. In this background, the average growing stock increased from 124m³ ha⁻¹ to 141m³ ha⁻¹ between 1990 and 2005 (Jactel *et al.*, 2009). These increments are projected to continue for the next decades. Under a scenario of increasing afforestation in Europe it becomes most relevant thus to understand what the best species to plant would be, considering climate change scenarios. Also, there is a need at European level to define policies regarding afforestation programs.

In Portugal forests cover 3 261 501ha, representing 35% of the continental territory, where 106 701ha is located in the archipelagos of Madeira and Azores. Overall, the Portuguese forest is mainly dominated by four tree groups of species: *Eucalyptus* spp. (26%), *Pinus pinaster* Ait. (23%), *Quercus suber* L. (23%) and *Quercus ilex* L. (11%) (ICNF, 2017). Thus, although *Eucalyptus* spp. plantations occupies large forest areas, forest cover in Portugal is still dominated by native species. However, this forest land use distribution may change in the future, due to several environmental, economic and social drivers. Among environmental drivers, climate and emergent pests and pathogens, will play a relevant role. Climate changes may force changings in forest land use, namely, to adapt to increase temperature and drought periods. Invasive pests and pathogens species may compromise the survival and productivity of some forest species. These changes will reflect themselves in

industry, such as by altering the utilized tree species and forest products. As an example, a decrease of maritime pine plantations has been observed in the last 10 years, from 805 100 ha in 2005 to 718 300 ha in 2015. This change was, in great part, due to the mortality caused by the pine wood nematode, *Bursaphelenchus xylophilus* (Steiner & Bühner) Nickle, being also responsible for dramatically affecting the wood chain and industries based on the maritime pine wood. Conversely, an area increase of the umbrella pine, *Pinus pinea* L., was observed in the same period, with a production rise of edible pine nuts with high commercial value. Governmental policies for afforestation programs and landowner's choice for new plantations must thus take in consideration the needs for adaptation to climate changes and biotic risks, as well as other environmental, economic, and societal pressures. In face of a changing forest use, we need experimental studies to help deciding on tree selection for future plantations. The REINFFORCE Infrastructure (Réseau INFrastructure de recherche pour le suivi et l'adaptation des FORêts au Changement climatique) was created to study the climate responses of tree species within the European Atlantic Region. It comprehends 38 arboreta planted between latitudes 37° and 57° N, all containing the same 33 tree species (Correia *et al.*, 2018). Each of the 33 species ideally would be represented by three mandatory provenances. The scope of this infrastructure was to allow a better understanding of the desired tree species capacity to cope with future climate changes, as a basis for future forest planning in European Atlantic regions. Six of the 38 arboreta were installed in Portugal, 3 three in mainland (Lisbon, Sintra, and Vila Real) and three in the Azores Island S. Miguel.

Since pre-historical times, forests have provided many valuable goods and services to human beings, including provision of wood, food, medicinal and other forest products as well as recreation, spiritual and aesthetic services, with high economic and social values (Bonan, 2008). All over the world, forests also provide major relevant ecosystem services, including biodiversity conservation, regulation of hydrological cycles, and protection of soil from erosion, carbon sequestration and climate effects mitigation. In fact, forests influence climate through physical, chemical, and biological processes with global planetary impacts, such as the atmospheric composition and the hydrological cycle (Bonan, 2008; Canadell & Raupach, 2008).

In the present climatic situation, it is particularly important to mention the influence of forest ecosystem on carbon sequestration (Canadell & Raupach, 2008). Since 1750, the atmospheric concentration of carbon dioxide (CO₂) has increased by 31%, due to fossil fuel combustion and land use change (Lal, 2004). Annually, terrestrial ecosystems remove nearly 3 billion tons of carbon, absorbing around 30% of all CO₂ emissions from fossil fuel burning and net deforestation, where forest ecosystems store about 45% of terrestrial carbon and contribute around 50% of terrestrial net primary production (NPP) (Bonan, 2008; Canadell & Raupach, 2008). Despite the climatic benefits associated with forests, there are also risks

associated to this carbon pool, namely the occurrence of forest fires and insect outbreaks. For example, in Canada, the recent increase of areas affected by wildfires and insect outbreaks passed these forest areas from being a CO₂ sink to source (Canadell & Raupach, 2008).

Regarding the hydrologic cycle, forests sustain it through evapotranspiration, which cools climate through feedbacks with clouds and precipitation. In this matter, there is a clear difference between forests from different climate zones. Tropical forests stand as the main contributor to evapotranspiration, hence causing high evaporative cooling, being followed by temperate forests with a moderate evaporative cooling, whereas boreal forests have a weak evaporative cooling contribution. Also, conifer forests have a lower ratio of evapotranspiration than deciduous broadleaved forests (Bonan, 2008).

Despite of its influence on climate, forests are also particularly sensitive to climate changes. Although tree species, like other organisms, are continuously adapting to the site conditions where they occur, the long-life span of trees does not allow for rapid adaptation to environmental changes (Lindner *et al.*, 2010). One mechanism of adaptation could be the expansion of tree species to other regions such as from South to Northern areas in Europe, as observed with some animal species (Feehan *et al.*, 2009). However, unlike animals, tree species have a limited migration capability. Thus, it is becoming more and more appealing the concept of assisted migration of plants, in which the species distribution changes in latitude and altitude would be facilitated by human direct activity (Vitt *et al.*, 2010).

Relationships between trees and environment are going to be affected by climate. Plant growth and phenology may change in function of climate, as well as pests and pathogens, which means that the forest composition is also at risk of changing. Understanding these drivers is most important to plan future forests. In the next sections we will address the expected impacts resulting from direct influence of climate on trees, and indirect consequences in phenology and herbivory.

1.2. Climate change effects on trees

Climate change is considered one of the main threats that mankind is going to face during the XXI century, since it brings large consequences to every part of ecosystems with consequent social, economic and environmental impacts.

Changing temperature and precipitation pattern and increasing concentrations of atmospheric CO₂ are likely to drive significant modifications in natural and planted forests (Kirilenko & Sedjo, 2007). In order to make decisions about forests, we must look to the last decades and analyse climatic data to understand changing trends and its effects on trees.

In the last century, the global average temperature has increased by 0.8°C. However, 0.6°C of that increment has happened just in the past 3 decades (Hansen *et al.*, 2006). This

means a drastic acceleration in temperature rising, representing an increment of +0.2°C per decade. This fact confirms that the European heatwave of 2003 was a drastic demonstration of the extent of impacts we need to expect more often in the future.

Being temperature a key ingredient for trees development, changing temperatures will cause several effects on trees. Specifically, temperature increase may cause an increase in tree growth for boreal and temperate regions since they are growing under their temperature optimum. Conversely, higher temperatures have shown a decrease in tree growth for tropical trees which are already growing on their temperature optimum (Way & Oren, 2010). In tropics, evergreen trees are taller and skinnier, having more foliage than roots. These trees reveal a less pronounced response to changing temperature than deciduous trees on temperate forests (Ryan, 2010). Physiologically, there is the risk of photorespiration being stimulated with higher temperatures and photosynthesis being inhibited by inhibiting the Rubisco activase, therefore not allowing for carbon fixation and thus not completing the Calvin-Benson cycle (Crafts-Brandner & Salvucci, 2000; Law & Crafts-Brandner, 1999). Respiration is also affected by rising temperatures in a direct proportion due to an exponential increase in leaves respiration, when temperatures increase to the trees optimum and beyond (Rennenberg *et al.*, 2006).

Also, precipitation at a global scale has increased by about 2% since the beginning of 1900 (Hulme *et al.*, 1998). Still, precipitation is not equally distributed on Earth's regions. In mid and high latitudes, for zones ranging from 30°N to 85°N, the average precipitation has increased between 7% and 12% over the 20th century. In the northern hemisphere, these increments tend to occur temporally distributed in an irregular way, mainly during autumn and winter. In low latitudes the opposite tends to happen, where a decade-long reduction in global precipitation was observed from the mid-80s to mid-90s, having lowest precipitation happened since 1995 (Dore, 2005). Regarding Europe, increases in precipitation from 10% to 40% were verified in its northern half, whereas in the southern half of Europe some regions have dried up to 20% (Dore, 2005). For example, in Spanish southern coast and Pyrenees, the number of days with precipitation have decreased by 50% and 30% respectively, between 1964 and 1993 (Romero *et al.*, 1998). Considering current data and looking from a worldwide point of view, it is expected longer and more intense drought periods in the Mediterranean basin as well as an increase in mean annual temperature, with fewer frost days per month (Dore, 2005).

Additionally, drought is known to affect trees development through increasing the diffusive resistances to CO₂ entry into chloroplasts, thus decreasing the efficiency of photosynthesis (Flexas *et al.*, 2004). The increase of these resistances will change the ratio of CO₂ and O₂, available for Rubisco, potentially leading to an increase in competition between photosynthesis and photorespiration. If drought occurs simultaneously with heat waves, it may have an exacerbated effect. In fact, respiration seems to be enhanced when drought and heat are combined which may be seen as a defence mechanism (Rennenberg *et al.*, 2006).

Lastly, the increasing concentration of CO₂, along with other gases (greenhouse gases), was proven to be the main factor for climate changing and global warming. Despite it, its concentration also impacts trees development. Since CO₂ is the unit element to photosynthesis, an increase in atmospheric CO₂ will drive an increase in photosynthesis activity and, therefore, enhance NPP (Luo *et al.*, 2004). This fact allows to conclude that, if CO₂ atmospheric concentration increases, the same will happen to trees growth although not in a continuously sustained way. In fact, Idso (1999), has shown a decline in biomass production over the years in CO₂-enriched systems. We now know that elevated levels of CO₂ can stimulate the sequestration of carbon (C) and nitrogen (N) to soil organic matter (SOM) and to long lived plants (such as trees). On another hand, there is also the progressive nitrogen limitation (PNL), which shows us that in an environment enriched with CO₂, N availability is going to be a constraint due to its replenishing being slower than its sequestration/exploitation by organic matter (Luo *et al.*, 2004). Due to PNL there are only two options, either new N inputs will exist to balance its loss, enabling a continuous growth for trees in the long-term. Otherwise, with elevated CO₂ concentration, there will only occur enhanced growth and C sequestration for the short-term.

1.3. Phenology

Phenology is the sequence of periodic events that happen in an animal or plant life cycle, which is usually triggered by environmental factors. Differences in phenology result from the adaptation of organisms to seasonal changes in environmental conditions and available resources, along with its genetics. By studying the different stages of development of organisms along the year, we conclude that phenology tends to optimize survival, reproduction and adaptive ability, presupposing climate stability. However, in face of climate changes, phenology may create maladaptation. It is, thus, of extreme importance to understand how climate affects tree phenology so in order to develop strategies to cope with it, in case of negative impacts are to be expected.

We know that phenology is highly sensitive to climate change (Richardson *et al.*, 2013). Several studies have demonstrated a correlation between earlier spring phenology in plants and rising temperatures in recent years (Cleland *et al.*, 2007). There is also an effect of the winter chilling and photoperiod on plant bud burst. Photoperiod is responsible for induction and release from buds dormancy in some plant species, while temperature modulates and triggers the visible progress of phenology (*e.g.* leaf unfolding) (Körner & Basler, 2010).

The increase in temperature also seems to lengthen the growing season, which may affect the exposition to insect pests and pathogens and also leaving plants more susceptible to extreme weather events, such as freezing (Shahin *et al.*, 2019). This outcome may bring

risks to local plant productivity and growth. In extreme scenarios it might come to cause differences in the community's composition.

Since tree species are adapted to the site where they evolved, different provenances shall have different characteristics and therefore, react differently to the climatic drivers and climate change. Also, we might expect that, the more sensitive a species is to a certain parameter, such as temperature, the faster will be the phenological differences in response to changes on that parameter. Species with an earlier leaf unfolding have a longer period with adult leaves, which may be positive in terms of productivity. However, this change may bring an increased risk to early spring frost damage on leaves (Leinonen & Hänninen, 2002). Further, a longer period of leaf exposition may be accompanied by a higher period during which the plant will be more susceptible to herbivory (Basset, 1991). In turn, leaf consumption by herbivores will reduce total leaf area which implies lower carbon assimilation through photosynthesis.

1.4. Herbivory

Herbivory is the action of consuming vegetation both below and above ground. Being an integral part of forest ecosystems, through their feeding activity herbivores impact the plant species composition, ecosystem function and socioeconomic value of forests (Ayres & Lombardero, 2000). Different pests and diseases have different life cycles and different modes of consumption. Each biotic agent attacks specific types of plants, being more or less specialized according to its host range. Still, most herbivores, even generalists, consume only coniferous or broadleaved trees. A further differentiation regards plant organs attacked. Most herbivores concentrate its activity in one part of the plant (roots, phloem under bark, trunk, branches, buds, leaves, flowers, and fruits/seeds). Clearly, there is a relationship between the plant phenology and the availability of certain plant organ to the herbivores, e.g. expanded leaves, buds, fruits, etc.

Regarding herbivory damage, we may also assume that in a certain location, plant species have been evolving in a way to improve their defence systems against the attacks of biotic agents. The other way around is also true, i.e., the biotic agents have been evolving to develop counter-adaptations to plants defences, improving their capability of extracting the needed resources from those plants. This is demonstrated by the "arms race" hypothesis (Kant *et al.*, 2007). Hence, when an exotic tree species is introduced in a new region, pests and pathogens could cause them much lesser herbivory damage than to native species (Branco *et al.*, 2015; Carlsson, Sarnelle & Strayer, 2009). We may thus expect that, compared to exotic species, native plant species will have a higher degree of consumption by herbivores, due to the high number of herbivore species, pests and pathogens, with co-evolutionary history

feeding on it. On the contrary, exotic species are new in the ecosystem and therefore have no associated biotic agents. Still, the opposite may also be true, generalist herbivores may expand their feeding range to new host plant species, which in turn may have not evolved specific defences against these organisms.

One way for plants to escape herbivores could be through mismatch on the phenological window. That is the plant tissues or organs development would not coincide with the period of activity of the herbivores (Singer & Parmesan, 2010; Dixon, 2003; Murali & Sukumar, 1993). Despite different concepts and actions, it is evident that phenology and herbivory are connected. If the herbivore reproduction and development occur outside its window of favourable food, such as the bud bursting period of the host plant for a bud feeder, there will be major consequences regarding its fitness (Visser & Both, 2005). Yet, apart from plant resources, herbivores have other requirements to complete its life cycle, such as temperature and habitat. Thus, the relationship between the plant and the herbivore, can be influenced by climate changes, either by influence on their phenology or indirectly by other factors. Also, climate may affect plant physiology, which indirectly will affect plants resistance to herbivores (Ayres, 1993), as well as the herbivores capability to complete its normal life cycle.

Due to the complexity of factors involved, there is a need for further studies to understand how trees will cope with climatic and biotic constraints in face of climatic and global changes in general.

1.5. Objectives of this study

In this work we propose to follow two arboretums belonging to the Reinforce network, located in Portugal, and assess differences in phenology and herbivory among species and provenances. It will be tested: i) whether phenology is dependent on provenance for selected *Quercus* species; ii) whether phenology has implications on insect herbivory; and iii) whether the species belonging to the native Portuguese flora are more or less exposed to damage by herbivores, than non-native species.

In each arboretum a minimum of 36 trees per species was supposed to be found, as well as a minimum of 3 provenances per species in each location. However, due to problems in the seedling production and seed availability, plantations were incomplete (Orazio *et al.*, 2014). Also, due to high mortality on the first four years only some species survived in each arboretum (Correia *et al.*, 2018). We will concentrate our study in a few species present in both arboreta with significant numbers of replicate plants.

Since provenances are originated from different climatic regions, this study enabled us to test the possibility of different provenances having different phenology as well as different herbivory.

2. Material and Methods

2.1. Study Sites

This study took place in two of the six Portuguese REINFFORCE arboreta, located in Lisbon and Sintra, respectively (Table 1). The site AR35 – Tapada da Ajuda, is placed inside a public property managed by Instituto Superior de Agronomia (ISA), being currently used for educational and research purposes. The site AR34 – Sintra, is located in Sintra-Cascais Natural Park being administered by Parques de Sintra – Monte da Lua (PSML). This site is divided in two separate parcels due to terrain obstructions, Tapada das Roças as “Parcel 1” and Tapada do Mouco as “Parcel 2”. Although the two sites are geographically near, about 23.2 Km distant in a straight line, the two sites greatly differ in their climatic variables, namely temperature and rainfall (Table 1).

Table 1: Study sites characterization.

Characteristics	Instituto Superior de Agronomia (Lisboa)	Parques de Sintra-Monte da Lua (Sintra)
Coordinates (Latitude; Longitude)	38°42'57.43"N; 9°11'32.27"W	Parcel 1: 38°46'54.20"N; 9°24'43.78"W. Parcel 2: 38°46'48.46"N; 9°24'22.76"W.
Slope, orientation	8%, East	19%, North-West
Altitude	106 m	400 m
Soil type	Leptosols, subtype vertic and bedrock of basic to ultrabasic volcanic rocks	Cambisols, subtype humic and bedrock of acid to intermediate plutonic rocks
Temperature (average)	Annual: 17.5°C. Coldest month: 7.2°C	Annual: 13.6°C. Coldest month: 5.4°C
Precipitation (annual average)	823 mm	1019 mm
Frost days	0	1

Adapted from Orazio *et al.*, (2014)

To simplify the monitoring of the sites and to avoid competition between fast growing species and slow growing species, seedlings were installed in 2012 following nested schemes:

- Broadleaves on one side and conifers on the other side
- Within conifers: pines and non-pines
- Within broadleaves: oaks and non-oaks

Currently, there are 13 species of conifers and 15 species of broadleaves in Tapada da Ajuda, and 15 species of conifers and broadleaves in Sintra (Table 2).

Table 2: Species list present in both arboretums, at the time of this study.

Site	Conifers	Broadleaves
Tapada da Ajuda	<i>Calocedrus decurrens</i> (Torr.) Florin. <i>Cedrus atlântica</i> (Endl.) Manetti ex Carriere <i>Cupressus sempervirens</i> L. <i>Pinus brutia</i> Ten. <i>Pinus caribaea</i> subs. <i>hondurensis</i> (Sénécl.) Silba <i>Pinus elliotii</i> Engelm. <i>Pinus nigra</i> Arnold. <i>Pinus peuce</i> Griseb. <i>Pinus pinaster</i> Ait. <i>Pinus pinea</i> L. <i>Pinus ponderosa</i> Douglas ex. C. Lawson <i>Pinus sylvestris</i> L. <i>Pinus taeda</i> L.	<i>Acer pseudoplatanus</i> L. <i>Betula pendula</i> Roth. <i>Castanea sativa</i> Mill. <i>Ceratonia siliqua</i> L. <i>Eucalyptus globulus</i> Labill. <i>Eucalyptus gundal</i> Hook. <i>Eucalyptus nitens</i> Maiden. <i>Fagus orientalis</i> Lipsky <i>Liquidambar styraciflua</i> L. <i>Quercus ilex</i> subsp. <i>rotundifolia</i> Lam. <i>Quercus petraea</i> Matt. <i>Quercus robur</i> L. <i>Quercus rubra</i> L. <i>Quercus shumardii</i> Buckland. <i>Quercus suber</i> L.
Sintra	<i>Calocedrus decurrens</i> (Torr.) Florin. <i>Cedrus libani</i> A. Rich. <i>Cunninghamia lanceolata</i> (Lamb.) Hook. <i>Cupressus sempervirens</i> L. <i>Larix decidua</i> L. <i>Pinus brutia</i> Ten. <i>Pinus elliotii</i> Engelm. <i>Pinus nigra</i> Arnold. <i>Pinus pinaster</i> Ait. <i>Pinus pinea</i> L. <i>Pinus ponderosa</i> Douglas ex. C. Lawson <i>Pinus sylvestris</i> L. <i>Pinus taeda</i> L. <i>Sequoia sempervirens</i> (D. Don) Endl. <i>Thuja plicata</i> Donn ex D. Don	<i>Acer pseudoplatanus</i> L. <i>Betula pendula</i> Roth. <i>Castanea sativa</i> Mill. <i>Ceratonia siliqua</i> L. <i>Eucalyptus globulus</i> Labill. <i>Eucalyptus gundal</i> Hook. <i>Eucalyptus nitens</i> Maiden. <i>Fagus orientalis</i> Lipsky <i>Liquidambar styraciflua</i> L. <i>Quercus ilex</i> subsp. <i>rotundifolia</i> Lam. <i>Quercus petraea</i> Matt. <i>Quercus robur</i> L. <i>Quercus rubra</i> L. <i>Quercus shumardii</i> Buckland. <i>Quercus suber</i> L.

2.2. Tree health monitoring

2.2.1. Arboreta health status

Health status monitoring was conducted targeting different objectives. Due to logistic difficulties some studies included only the Lisbon arboretum. In particular we aim at analysing i) tree survival in the Lisbon arboretum, ii) health status of all tree species in Lisbon arboretum, iii) assessment of the most important defoliator of pine trees, in Lisbon and Sintra arboretums iv) assessment of seasonal variation on *Quercus* trees herbivory in Lisbon arboretum, and v)

comparison of *Quercus* trees herbivory, between Lisbon and Sintra arboretums. The description of each of these activities is done below.

Survival

The number of plants currently surviving at 6-years old, after plantation, was registered and then used to estimate survivorship between 0 and 6 years after plantation.

Pests and pathogens

Pests and pathogens were assessed using the health protocol defined for Reinfforce network (Lucchio *et al.*, n.d.), with some adjustments (Tables 3 and 4). The complete health assessment was done in Lisbon arboretum, in June 2019, when the leaves of broadleaved trees were fully expanded.

Table 3: Health assessment categories adapted from Reinfforce arboretum field manual.

Damage	Classes	Code
Crown mortality (proportion of the crown affected, branch or shoot mortality)	5 classes of mortality: 0% (null), 1-10% (low), 11-50% (moderate), >50% (high), 100% (dead)	CrownM
Crown defoliation (proportion of the crown affected by leaf/needle consumption or damage)	6 classes of defoliation: 0% (null), 1-10% (insignificant), 11-25% (low), 26-50% (moderate), >50% (high), 100% (leafless)	CrownD
Crown discolouration (proportion of the crown affected by abnormal colour (yellow, red, brown))	6 classes of discoloration: 0% (null), 1-10% (insignificant), 11-25% (low), 26-50% (moderate), >50% (high), 100% (discoloration)	CrownC
Biotic and abiotic agents	Presence / absence of a particular agent in a predefined list of 20 types	

Table 4: Type of damage, adapted from Reinfforce arboretum field manual.

9	Forest Herbivore Types
9.1	Chewers
9.2	Gall Makers
9.3	Leaf-miners
9.4	Skeletonisers
9.5	Leaf-rollers and tiers
9.6	Sap Feeders (honeydew, fumagine)
9.7	Shoot Deformation (shoots, moth)

9.8	Stem / Bark Borers (dust, resin, holes)
9.9	Mammal Grazer
6	Forest Disease Types
6.1	Rust
6.2	Mildew
6.3	Leaf Necrosis
6.4	Red Bands (needle cast)
6.5	Canker
6.6	Stem / Shoot Necrosis
3	Abiotic Types
3.1	Drought
3.2	Frost
3.3	Wind
3.4	Mechanical Damage
3.5	Fire

2.2.2. Susceptibility of pine species to the pine processionary moth

The pine processionary moth, *Thaumetopoea pityocampa* (Den. & Schiff.) (Lepidoptera, Thaumetopoeidae) is the most important herbivore of pine trees in Mediterranean countries. Since the arboreta contains different pine tree species, it was of interest to see specifically the presence of *T. pityocampa* nests, which can rapidly become a problem to forest management. In order to test differences in tree susceptibility to this defoliator, we assessed its presence on the pine species present on the two Reinforce arboretums located in Lisbon and Sintra.

The pine processionary moth produces larval nests during mid-Winter. By January-February the larvae abandon the nests and start descending the tree to pupate in the soil. Therefore, the survey for this specific pest could not follow the general methodology. In this case, we counted nests from January to mid-February 2019, when the pine processionary moth nests were still visible and the larvae have not yet abandon them.

2.2.3. *Quercus* herbivory along spring season

We conducted health status assessments from March to June 2019, on two *Quercus* species trees (*Q. robur* and *Q. rubra*) present in Tapada da Ajuda arboretum in order to relate herbivory with phenology. These assessments were done on the same plants where phenological studies were conducted (Table 5).

To note that, *Q. robur* (also known as European oak) is a European native tree species, whereas *Q. rubra* (also known as American oak) is an exotic tree species, native to North America.

2.3. Phenology

Phenology was observed on tree provenances belonging to *Q. robur*. and *Q. rubra*. Data collection was made according to phenology protocols created by the REINFFORCE network, (Lucchio *et al.*, n.d.). Observations were conducted from the end of February till May, when all trees had its leaves fully expanded. In this study, seven different provenances were observed, five from *Q. robur*. and two from *Q. rubra*.

The codes for each provenance were used as established for REINFFORCE network (Table 5).



Table 5: Provenance codes, for each species, and respective locations and number of individuals.







Species	Provenance Code	Location	Number of Individuals
<i>Quercus robur</i> L.	QURO-FRAN	France	9
	QURO-PAGO	Litoral Vasco-Navarro (E05), Spain	12
	QURO-POSA	Posavina, Croatia	6
	QURO-SUDO	Sud Ouest, France	1
	QURO-UNIT	New Forest, Hampshire, United Kingdom	14
<i>Quercus rubra</i> L.	QURU-FEST	Rubra (EST902), France	2
	QURU-VANA	(ES06) Litoral Vasco, Spain	4

Observations were made in every assessed individual, concerning the terminal buds present in the four pre-selected shoots, each one directed to a cardinal point.

To be able to correctly accompany bud bursting and leaf growth, the REINFFORCE infrastructure created an adaptation from the BBCH scale to the arboreta network from which I will only use the following 8 stages, where no stem elongation has happened (Table 6):

Table 6: Phenology development stages and respective description with visual support. Photos author: André M. Pinto.

Stage	Description	Photo
0	Winter dormancy	
1	Beginning of bud swelling, inducing a size and colour change	

7	The bud has produced green tips, starting to burst	
9	Retaining its bud shape, leaves have appeared and start to grow	
11	The first leaves have separated, still with a small size	
13	The leaves are now completely unfolded	
15	The leaves have reached their final size, but are still pale green	
19	The leaves are now fully matured	

To compare differences on leaf phenology development between the native and exotic species, and provenances, we used stage 13 as a differentiator level. We used this stage as it represents the phase where plants already have completely unfolded leaves

2.4. Data Analysis

An exploratory data analysis was done using Microsoft Office Excel. Statistical analysis was performed using IBM SPSS statistics for windows. For all analysis we considered a significance level of $\alpha = 0.05$.

To assess for tree mortality between native and exotic tree species, and also between broadleaved and conifer tree species, we used generalized linear models with origin (native, non-native) and type as predictor variables.

To test if damage differed among species and provenances, and between *Quercus* species from the two arboretums we used generalized linear models with Binomial distribution considering the predictors species, provenance and location. Each type of damage was considered separately.

To assess the influence of pine tree species in the occurrence of the pine processionary moth, we used a fisher's exact test.

To compare phenology curves, number of days of development until stage 13, among *Quercus* species and among *Quercus* provenances we used survival analysis with the Kaplan-Meier estimator and Log-rank test.

For multiple comparisons we used Tukey post-hoc HSD test.

3. Results

3.1 Health assessment

3.1.1. Survival

From the survival data we may conclude that survival ranged between 0% and 85% for all the species in the arboretum (Table 7 & 8). From the totality of 1532 plants, with which Lisbon arboreta was created, only 629 (41%) have survived in the 6 years of existence. Only five tree species had mortality levels below 35%, *P. pinea*., *Q. ilex subs. rotundifolia*, *C. sempervirens*, *P. brutia* and *Q. shumardii* (Figure 1). In opposition, *P. peuce*, *P. caribaea subs. hondurensis*, *C. atlantica* and *C. decurrens*, all had 100% of mortality.

Table 7: Lisbon arboretum survival - broadleaved species.

Species	Initial N.	Ending N.	0 to 6 y.o. survival (%)
<i>Q. ilex subs. rotundifolia</i>	72	52	72.2
<i>Q. shumardii</i>	12	8	66.7
<i>E. globulus</i>	12	6	50
<i>Q. suber</i>	285	131	46
<i>C. siliqua</i>	84	38	45.2
<i>Q. petrea</i>	36	15	41.7
<i>Q. robur</i>	149	59	39.6
<i>Q. rubra</i>	26	7	26.9
<i>E. gundal</i>	12	3	25
<i>E. nitens</i>	12	3	25
<i>B. pendula</i>	115	16	13.9
<i>F. orientalis</i>	24	3	12.5
<i>A. pseudoplatanus</i>	36	3	8.3
<i>C. sativa</i>	43	2	4.7
<i>L. styraciflua</i>	24	1	4.2

Table 8: Lisbon arboretum survival – coniferous species.

Species	Initial N.	Ending N.	0 to 6 y.o. survival (%)
<i>P. pinea</i>	143	122	85.3
<i>C. sempervirens</i>	36	25	69.4
<i>P. brutia</i>	37	25	67.6
<i>P. pinaster</i>	179	93	52
<i>P. ponderosa</i>	22	8	36.4
<i>P. taeda</i>	24	5	20.8
<i>P. elliotii</i>	12	1	8.3
<i>P. nigra</i>	41	2	4.9
<i>P. sylvestris</i>	46	1	2.2
<i>C. decurrens</i>	5	0	0

<i>C. atlantica</i>	3	0	0
<i>P. caribaea subs. hondurensis</i>	6	0	0
<i>P. peuce</i>	36	0	0

Although exotic species had an average value of 75.9% ± 5.4 of mortality and native species had an average of 56.8% ± 8.6, this difference was not significant ($\text{Chi}^2 = 3.469$; p-value = 0.063). However, results differ when exotic and native species are compared at genus level. For pines, mortality was significantly higher on exotic pine species in comparison with native ones ($\text{Chi}^2 = 48.06$; p-value < 0.001). On the other hand, there was no significant difference between exotic and native oak species ($\text{Chi}^2 = 0.516$; p-value = 0.472).

No significant differences were found on survival, also, according to the type of tree in general, i.e. broadleaves versus conifers. Broadleaved species had an average survival of 63.8% ± 6.6 and conifers 67.6% ± 7.2 ($\text{Chi}^2 = 0.1948$; p-value = 0.659).

The most used species in Portuguese forestry, *E. globulus*, *P. pinaster* and *Q. suber*, had relatively high mortality values of 50%, 48% and 54%, respectively.

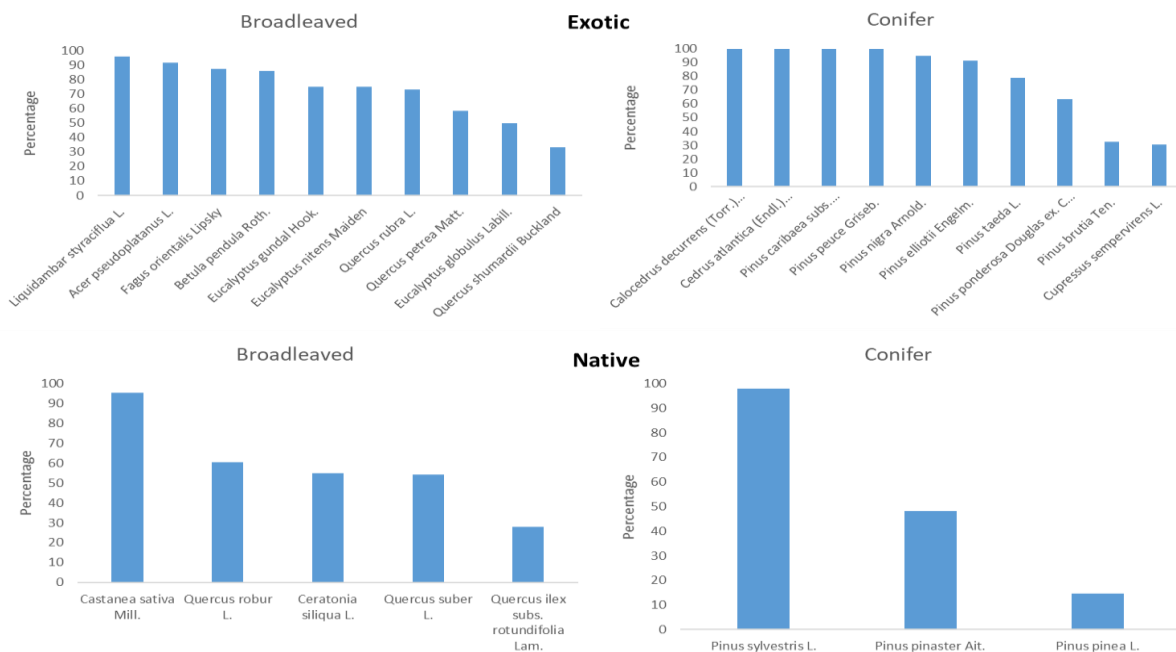


Figure 1: Species mortality rates, in Lisbon arboretum.

Due to mortality, trees distribution by genus and species was uneven. In Lisbon arboretum *Quercus* sp. is the most present counting with 272 individuals, being followed by *Pinus* sp which has 257 individuals and then by *Ceratonia* sp. and *Cupressus* sp., counting with 38 and 25 individuals each. The remaining genus had less than 20 individuals, including

Betula sp. (16 individuals), *Eucalyptus* sp. (12 individuals), *Fagus* sp. and *Acer* sp. (3 individuals each), *Castanea* sp. (2 individuals) and *Liquidambar* sp. (1 individual).

3.1.2. *Quercus* species assessment on the two arboretums

When comparing herbivory damage, on both arboretums, Tapada da Ajuda (Lisbon) and Parques de Sintra-Monte da Lua (Sintra), we may observe that the differences between both arboretums are significant in 8 out of 13 damage types, along with the percentage of individuals affected by them (Table 9). Leaf miners, shoot deformations, necrosis and drought values were much reduced on both arboretums and thus differences were not significant. Drought was only verified in Sintra, in 2 plants. Sap feeders, leaf necrosis and stem and shoot necrosis, were only verified in Lisbon, in a few individuals.

For the other types of damage, chewers, skeletonisers and mildew were higher in Lisbon than in Sintra arboreta. On the other hand, gall makers were much more frequent in Sintra than in Lisbon (Table 9).

Table 9: Test results on defoliation damage and the verified types of herbivory, regarding *Quercus* from both arboretums.

Damage types	Lisbon average % ± SE	Sintra average % ± SE	Wald Chi-Square	Sig.
Defoliation	18.25 ± 1.312	5 ± 1.474	45.096	< 0.001
Chewers	100 ± 0	13 ± 5.5	250.8	< 0.001
Gall Makers	2 ± 2.1	74 ± 6.1	92.744	< 0.001
Leaf Miners	67 ± 6.8	82 ± 6.3	2.591	0.108
Skeletonisers	67 ± 6.8	8 ± 4.4	52.79	< 0.001
Leaf Rollers & Tiers	6 ± 3.5	29 ± 7.4	7.766	0.005
Shoot Deformation	2 ± 2.1	5 ± 3.6	0.582	0.446
Rust	56 ± 7.2	32 ± 7.5	5.629	0.018
Mildew	60 ± 7.1	3 ± 2.6	59.031	< 0.001

3.1.3 Lisbon arboretum

At genera level

By comparing defoliation and decolouration degree, at tree genus level, we may observe higher defoliation damage for *Quercus* sp. and *Fagus* sp., whereas discoloration was higher for *Ceratonina* sp. (Figure 2).

Concerning the two most used genera, in Portuguese forestry, *Eucalyptus* sp. had an average discoloration and defoliation values of 6.3% ± 1.9 and 2.5% ± 1.8, respectively, as

for *Pinus sp.*, the average values were $1.1\% \pm 0.4$ and $0.84\% \pm 0.4$, for discolouration and defoliation respectively.

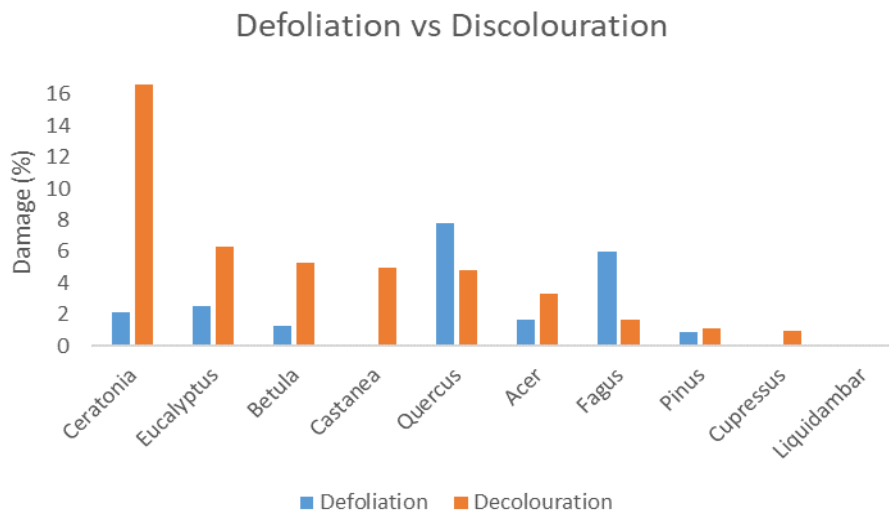


Figure 2: Comparison between defoliation and discolouration, in percentage, along the studied genus present in Lisbon arboretum.

Here, the predominant herbivory and damage types were chewers and sap feeders for pest types, leaf necrosis and red bands for pathogen types, and mechanical damage for abiotic types of damage (Figure 3 and Annex 1).

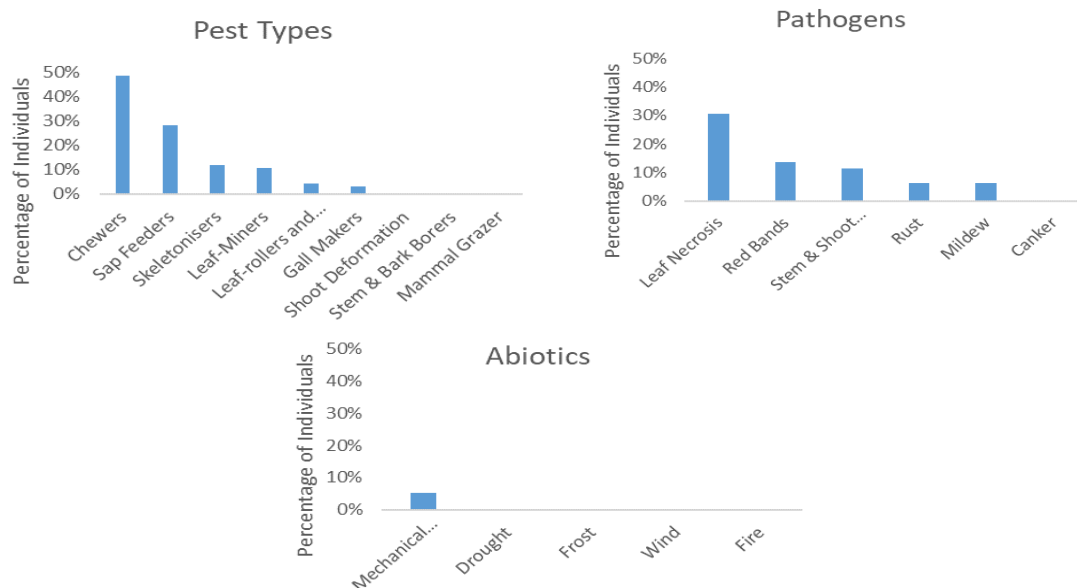


Figure 3: Percentage of every assessed damage type, in Lisbon arboretum.

While assessing damage types we observed they were highly genus specific. Canker and drought occurred only in *Pinus sp.*, whereas chewers, skeletonisers, leaf-miners, rust and mildew occurred mostly on *Quercus sp.* (Figure 4).

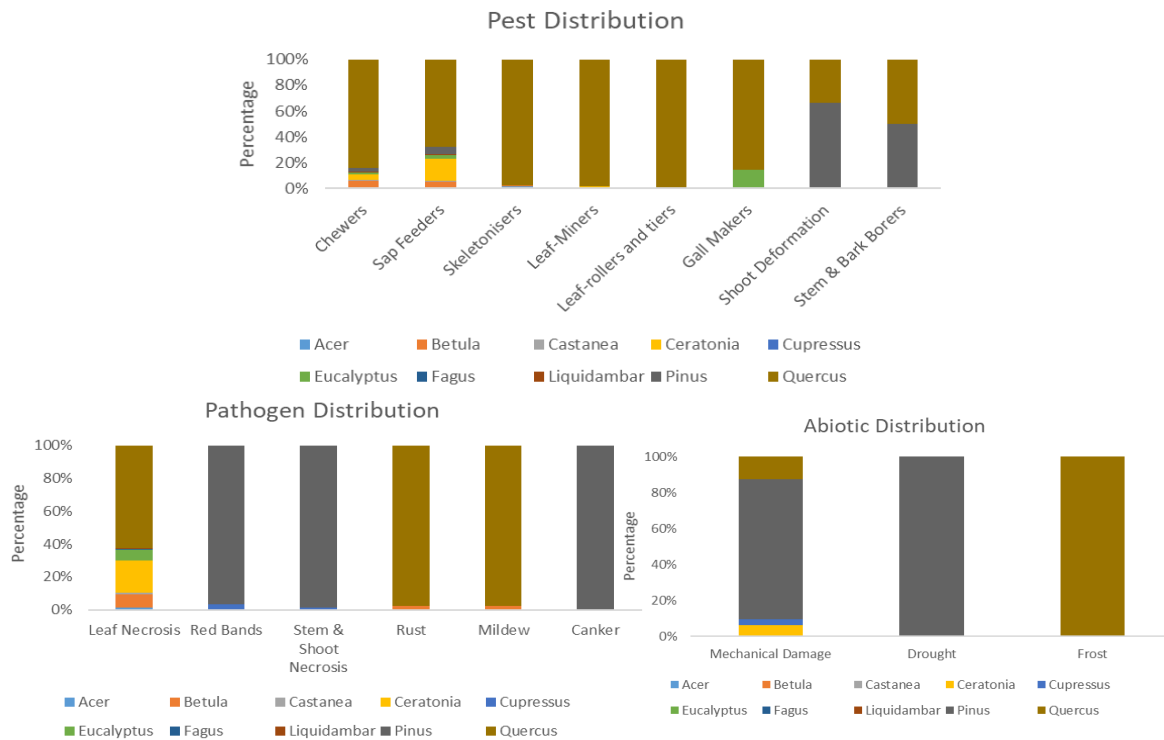


Figure 4: Damage distribution in percentage, by the ten studied genera.

Among broadleaves, *Quercus* sp. was the only affected genus by shoot deformation, stem and bark borers, and frost. This tree genus also had the highest number of individuals affected by sap feeders and leaf necrosis being followed by *Ceratonia* sp.. These damage types only existed sporadically on *Betula* sp. (3.9% and 7.8%, respectively for sap feeders and leaf necrosis), and *Eucalyptus* sp. (2.8% and 6.3%, respectively). Gall makers only affected two genera, being higher in *Quercus* sp. (85.7%) than in *Eucalyptus* sp. (14.3%).

Among conifers, *Pinus* sp. was the only genus affected by shoot deformation, stem and bark borers, and drought. Additionally, it had a high number of individuals affected by red bands and stem and shoot necrosis, whereas *Cupressus* sp. was only sporadically affected by these damage types (3.4% and 1.4%, respectively). Mechanical damage was mostly observed in *Pinus* sp., with 78.1% damaged individuals identified. The remaining genera showed no, or little signs of damage.

At species level

Comparing defoliation signs in broadleaved species, the exotic ones had an average defoliation damage lower than the native ($3.9\% \pm 2.5$ and $5.0\% \pm 0.7$, respectively). In coniferous species, the exotic ones had $1.2\% \pm 0.3$ of defoliation damage, which is three times higher than the defoliation damage observed on native species. When comparing discolouration signs, in broadleaved species, exotics presented an average damage value of

3.8% ± 2.4, equivalent to half of damage in the native species. However, in coniferous species, exotics had an average 0.8% ± 0.8 of damage, while coniferous native species had an average damage of 0.6% ± 0.2.

Despite the values above, some outliers existed (Figure 5).

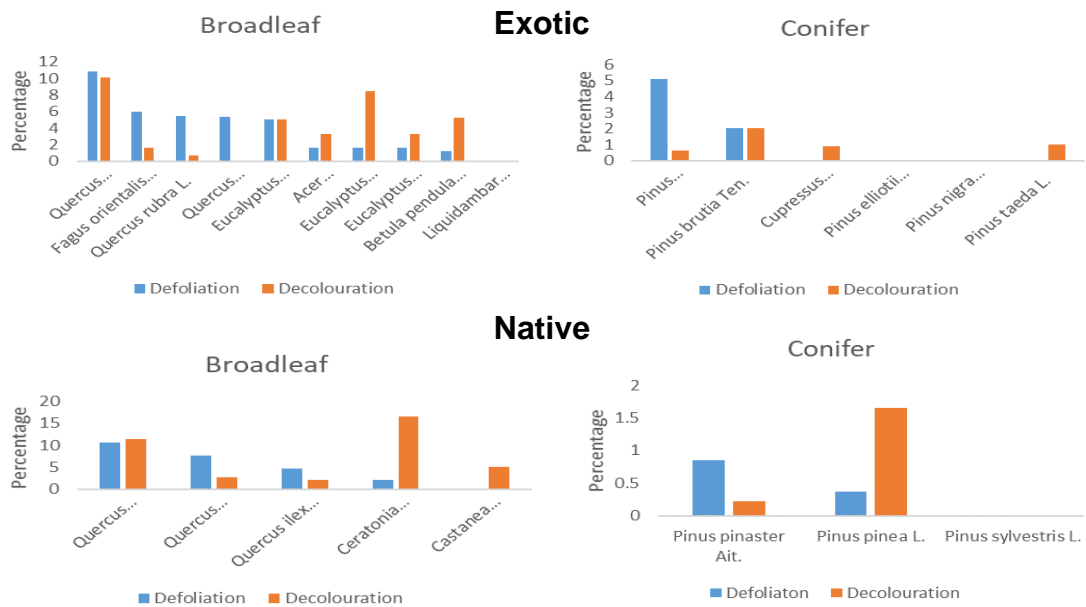


Figure 5: Comparison on defoliation and discoloration damage, in Lisbon arboretum species.

The highest average value of defoliation damage was verified in the exotic broadleaved species *Q. petraea*, with 10.9 % ± 1.6, whereas the highest average discoloration value was observed in the native broadleaved species *C. siliqua*, with 16.6% ± 1.

Regarding the most used species in Portuguese forestry, *Q. suber* had the highest defoliation average (7.6% ± 0.6), being followed by *E. globulus* and *P. pinaster* (1.7% ± 2.6 and 0.85% ± 0.3, respectively). In discoloration, *E. globulus* presented the highest value of 8.5% ± 2.6, followed by *Q. suber* and *P. pinaster* (2.8% ± 0.6 and 0.22% ± 0.3, respectively).

3.1.4 Susceptibility of pine species to the pine processionary moth

In Tapada da Ajuda, the pine processionary was observed mainly on two native pine species, *P. pinaster* and *P. pinea* (Figure 6). In Sintra arboretum no nest was encountered, it was assumed the inexistence of this pest in the arboretum.

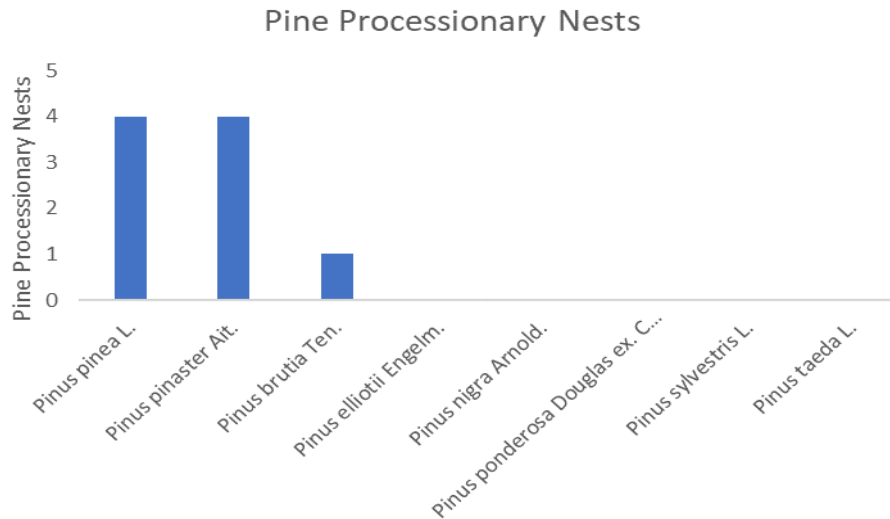


Figure 6: Pine processionary nest counting, in Lisbon arboretum.

The totality of the nests was found in only three pine tree species, from which *P. pinaster* and *P. pinea* were the most damaged, presenting the same number each. When assessing the presence of nests, their location was also registered. All of them were present on the upper part of the tree crown, from middle to top, and were always facing south.

We found no significant differences between pine species in terms of pine processionary occurrence among the affected tree species (Fisher's Exact Test = 0.448, $p = 0.892$).

3.1.5 Oaks damage variation along season

3.1.5.1 Species damage

The temporal change of foliage damage through spring season showed a linear increase of damage for both species *Q. rubra* and *Q. robur* (Figure 7). However, the increase rate was higher for *Q. robur*, attaining in June about 20% defoliation. This value is equivalent to the quadruple of taken damage by the exotic species *Q. rubra*.

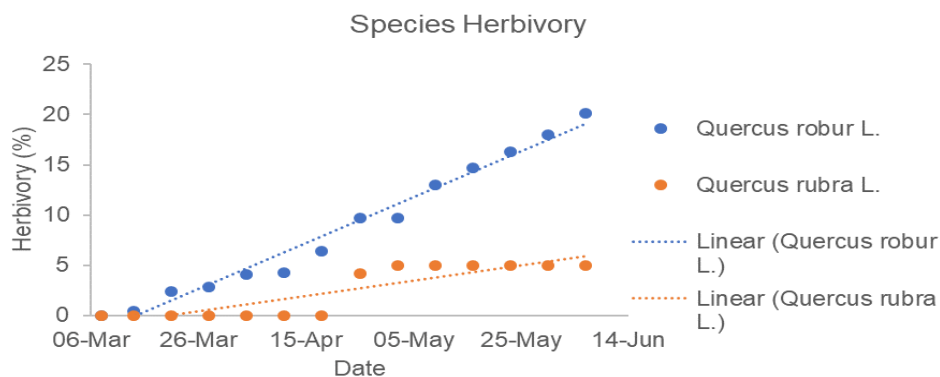


Figure 7: Herbivory damage on the two studied *Quercus* species, along the observation period.

The differences observed between the native species and the exotic species, regarding their respective herbivory damage was found to be significant ($\text{Chi}^2 = 9.799$; $\text{df} = 1$; $\text{p-value} = 0.002$). Despite the difference in herbivory damage, it was still unclear which pest and pathogens feeding guilds would most account for the observed damage. Analysing the types of damage, it is clear that chewers affected more individuals, followed by leaf-miners and skeletonisers (Figure 8). Stem and bark borers, mammal grazers, red bands and canker were not observed, as well as any of the abiotic types of damage.

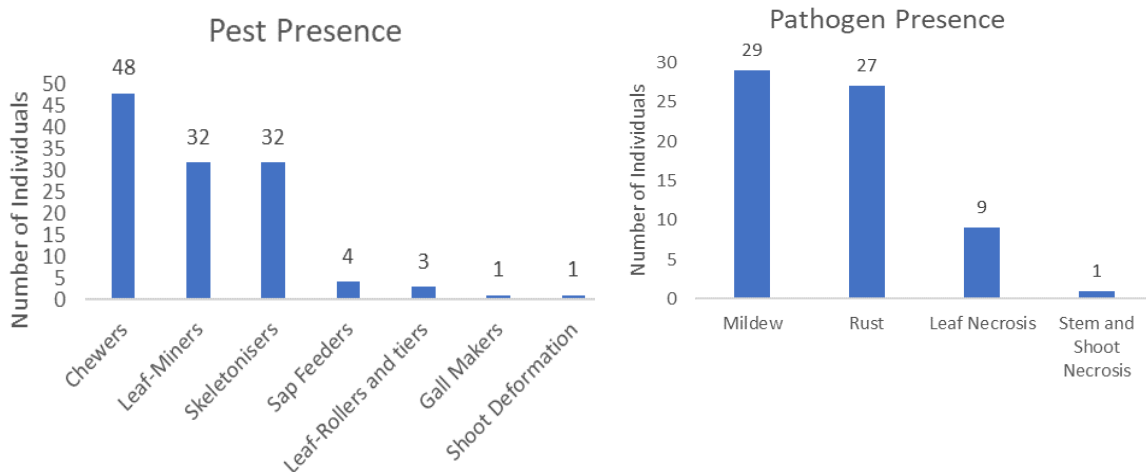


Figure 8: Verified quantities of pests and pathogens in Lisbon arboretum.

Regarding pests, chewers affected 100% of the individuals, of the two studied oak species. Two from major defoliators were identified. The first one was a weevil belonging to the *Orchestes* genus and was found from the 28th of March onwards. The second one was a beetle, *Lachnaia tristigma* (Lacordaire) (Coleoptera, Chrysomelidae). Chewers were followed by leaf miners and skeletonisers which affected 66.7% of the studied oak individuals. Concerning pathogens, mildew was the most present affecting 60.4% of the oaks analyzed, being followed by rust, which affected 56.2%.

Only five of the twenty different damage types were influenced by oak species. Regarding pests, differences were found for skeletonisers and sap feeders, which only affected the European oak (*Q. robur*). Regarding pathogens, rust, mildew and leaf necrosis, mostly affected the European oak individuals (Table 10).

Table 10: Overall test results on the verified types of herbivory, regarding species influence.

Damage Type	Wald Chi-Square	Sig.
Gall Makers	1.024	0.311
Leaf Miners	3.464	0.063
Skeletonisers	134.4	< 0.001

Leaf Rollers & Tiers	3.231	0.072
Sap Feeders	4.421	0.035
Shoot Deformation	1.024	0.311
Rust	75.6	< 0.001
Mildew	93.692	< 0.001
Leaf Necrosis	11.455	0.001
Stem & Shoot Necrosis	1.024	0.311

3.1.5.2 Provenance damage

In all the studied provenances, there was a linear increase in damage through spring season (Figure 9). Nonetheless, the rate of increase varied, where QUROPAGO got the highest value around 34%, correspondent to almost seven times the damage value of QUROFEST, QUROVANA and QUROSUDO, which had the lowest damage value.

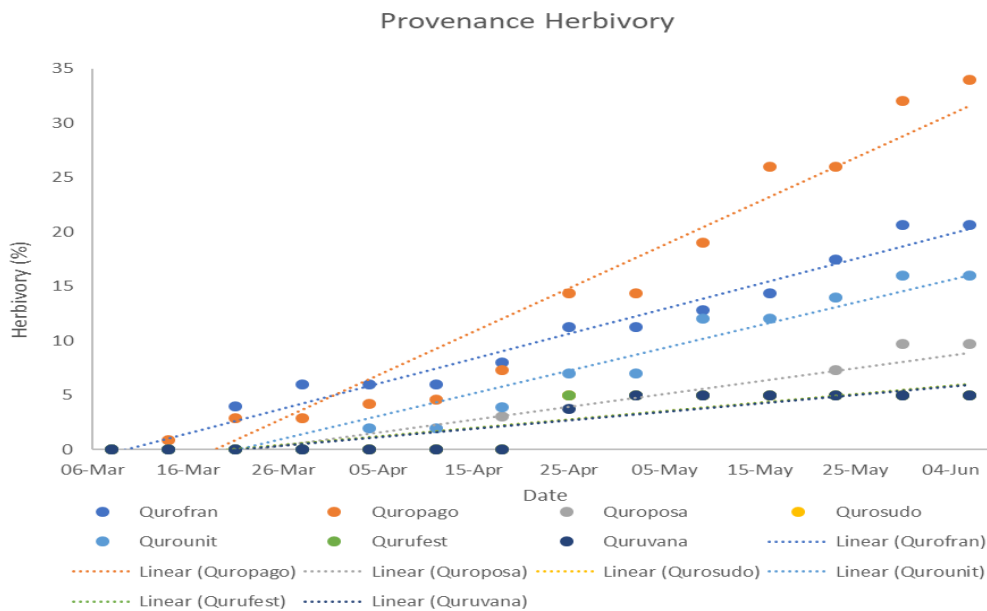


Figure 9: Herbivory damage, along the observation period, caused to the seven analysed Quercus provenances.

QUROPOSA and QUROUNIT had an average value of about 10% and 16%, respectively, being followed by QUROFRAN with around 21% of herbivory damage. In the same observation period, registered in June, it was proven that herbivory damage is influenced by provenance ($\text{Chi}^2 = 57.945$, $p\text{-value} < 0.001$).

Afterwards, it was assessed which provenances differed regarding their herbivory damage values (Table 11). QUROPAGO (from litoral Vasco-Navarro, in Spain) was shown to differ the most and QUROFRAN (from France) which, by very few, was found similar to

QUROUNIT (from New Forest Hampshire, in UK). All the remaining appeared to be similar, except for QUROUNIT and QURUVANA (from (ES06) litoral Vasco, in Spain).

Table 11: Pairwise comparison test of estimated mean values of herbivory damage, regarding each Quercus species provenance. * - the mean is significant at a 0.05 level.

(I) Prov.	(J) Prov.	Mean Dif. (I-J)	Std. Error	Sig.	95% Wald C.I.	
					Lower	Upper
Fest	Fran	-16.78*	6.349	0.008	-29.22	-4.33
	Pago	-26.33*	6.203	< 0.001	-38.49	-14.18
	Posa	-4.33	6.631	0.513	-17.33	8.66
	Unit	-10.21	6.139	0.096	-22.25	1.82
	Vana	0	7.033	1	-13.78	13.78
Fran	Pago	-9.56*	3.581	0.008	-16.57	-2.54
	Posa	12.44*	4.28	0.004	4.06	20.83
	Unit	6.56	3.47	0.059	-0.24	13.36
	Vana	16.78*	4.88	0.001	7.21	26.34
Pago	Posa	22.00*	4.061	< 0.001	14.04	29.96
	Unit	16.12*	3.195	< 0.001	9.86	22.38
	Vana	26.33*	4.689	< 0.001	17.14	35.52
Posa	Unit	-5.88	3.963	0.138	-13.65	1.89
	Vana	4.33	5.242	0.408	-5.94	14.61
Unit	Vana	10.21*	4.604	0.027	1.19	19.24

In the Fisher's exact test for provenance influence on the occurrence of damage types (Table 12), it was shown that leaf-miners and skeletonisers were most influencing, and from pathogen types, rust and mildew were also influencing.

Table 12: Fisher's exact test on the verified types of herbivory, regarding provenance influence

Damage Type	Fisher's Exact	Sig.
Gall Makers	6.132	0.447
Leaf Miners	11.576	0.022
Skeletonisers	22.164	< 0.001
Leaf Rollers & Tiers	3.995	0.582
Sap Feeders	5.234	0.286
Shoot Deformation	6.943	0.255
Rust	11.702	0.023
Mildew	13.355	0.01
Leaf Necrosis	2.403	0.875
Stem & Shoot Necrosis	5.249	1

Gall makers were only observed in QUROFRAN provenance, shoot deformation in QUROPOSA and stem and shoot necrosis in QUROUNIT but always in low numbers (Figure 10).

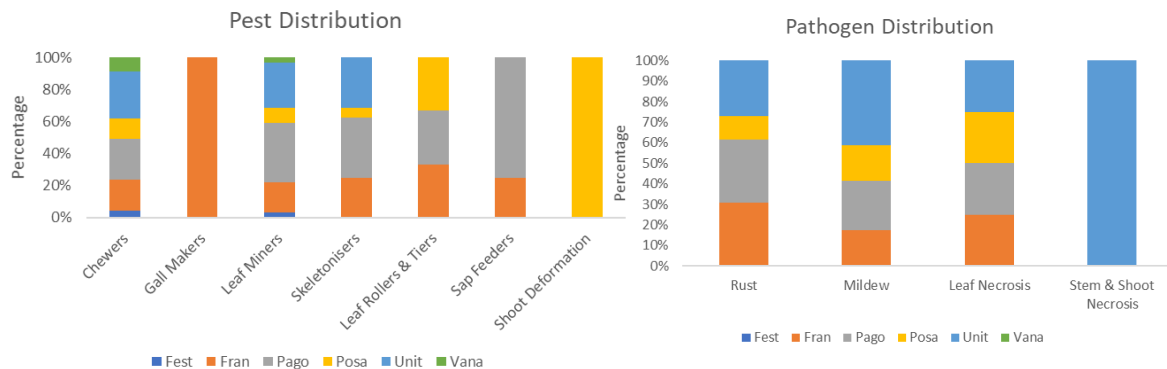


Figure 10: Damage distribution in percentage, along six of the seven assessed *Quercus* species provenances.

Concerning pest distribution along provenances, QUROPAGO was the most attacked, 100% of its individuals were damaged, followed by QUROFRAN and QUROUNIT, being these three the most affected by most of the verified pest types. Concerning pathogen distribution along provenances, QUROUNIT was the most damaged, being followed by QUROFRAN and QUROPAGO.

3.2. Phenology assessment

3.2.1. Differences among provenances

Significant differences were found on the phenology development, among provenances (Figure 11). The first provenance completing the phenology control was QURO-SUDO needing only an average of 87 Julian days to complete 100% of leaf development. Then came QUROPAGO and QUROFRAN, with 90 and 92 Julian days, respectively, followed by QUROUNIT, requiring an average of 96 Julian days and QUROPOSA, with a total of 104 Julian days. QURUFEST and QURUVANA, both required 114 Julian days to complete full leaf development.

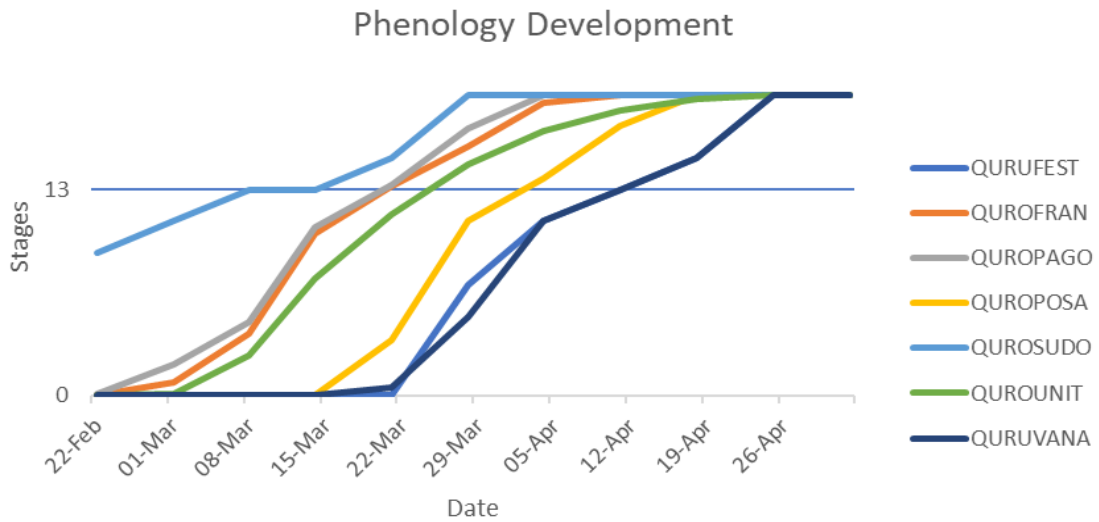


Figure 11: Phenology development average values along the study period, by species provenance.

Native vs Exotic species phenology

When comparing the average number of julian days that both *Q. robur* (European oak) and *Q. rubra* (red oak) took to reach their correspondent leaf development stage 13, we observed for the native species 82 ± 1 Julian days and 100 ± 0 Julian days for the exotic species. The native species, *Q. robur*, had a significant earlier bud bursting, when compared to the exotic species, *Q. rubra* (log-rank test $Q(1) = 20.325, p < 0.001$).

Provenance phenology

Provenance QUROSUDO reached development stage 13 first, being followed by QUROPAGO and QUROFRAN (Table 13). Due to the different attained mean values among provenances, differences were also significant (log-rank test, $Q(1) = 54.617, p = < 0.001$).

Table 13: Kaplan-Meier method, mean and median estimated values for phenology development stage 13 to be achieved, by the studied provenances.

Provenance	Mean \pm SE	Median \pm SE
QURUFEST	100 \pm 0	100 \pm 0
QURUFRAN	79.2 \pm 1.4	80 \pm 1.1
QUROPAGO	78.3 \pm 1.5	80 \pm 0.8
QUROPOSA	91.5 \pm 1.7	91 \pm 2.4
QUROSUDO	67 \pm 0	67 \pm 0
QUROUNIT	83.8 \pm 2.1	80 \pm 2.2
QUROVANA	100 \pm 0	100 \pm 0
Overall	84.2 \pm 1.3	80 \pm 1.2

Afterwards, we assessed the group differences (Annex 2), where homogeneous subsets were able to be created, according to their similarities (Table 14), and also from which it was shown that provenance QUROPAGO and QUROFRAN differed the most from provenances QURUFEST and QURUVANA, whereas QUROPOSA differed from QUROPAGO and QUROFRAN.

Table 14: Homogeneous subsets, according to provenance respective phenology average values (mean julian days).

Homogeneous Groups				
Provenance	N	Subset for alpha = 0.05		
		1	2	3
QUROPAGO	12	78.3		
QUROFRAN	9	79.2		
QUROUNIT	14	83.8	83.8	
QUROPOSA	6		91.5	91.5
QURUFEST	2			100.0
QURUVANA	4			100.0
Sig.		0.638	0.266	0.177

Provenance QURUFEST and QURUVANA appeared to be identical, with a comparison significance value of $p = 1$.

3.2.2. Phenology influence on herbivory

When phenology was compared with herbivory (Figure 12), a relationship was evident between the leaves development and the received respective damage by herbivores. Here, the earliest provenances in phenology have taken the most damage caused by herbivory.

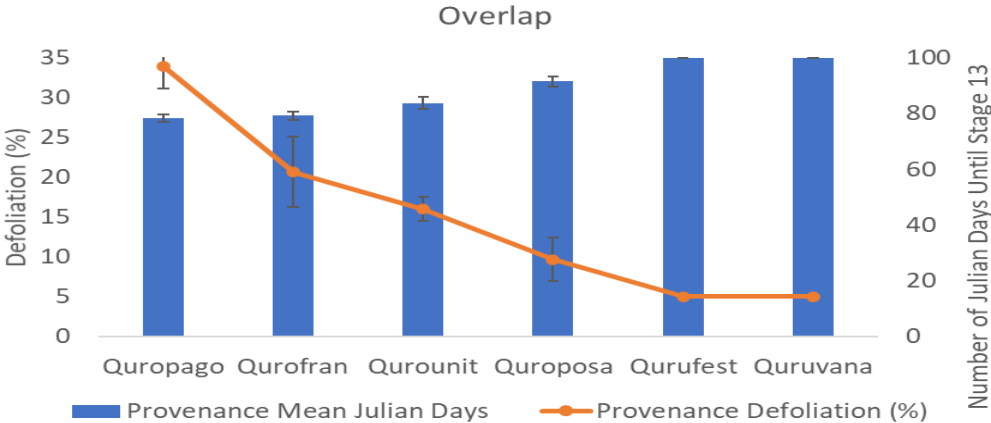


Figure 12: Overlap of provenance needed mean julian days, to reach stage 13, with provenance mean defoliation values.

The relation between phenology development and herbivory was shown to be almost perfect following an exponential trend with an R-squared value of 0.9667 (Figure 13). The Wald test value ($\text{Chi}^2 = 18.37$, $p < 0.001$) confirmed that phenology influenced the amount of herbivory damage.

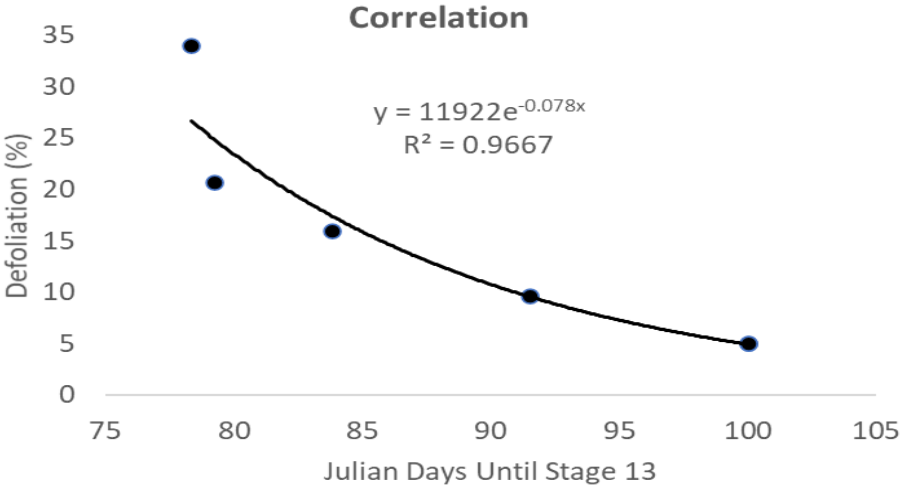


Figure 13: Correlation between the mean defoliation value of the studied provenances and their respective needed julian days until the development stage 13 was reached.

4. Discussion

Survival

On overall, mortality did not differ between exotic and native tree species. Still, differences varied with tree genus. Exotic pines had higher mortality when compared to native ones. This result could be justified by most of these pine species being not adapted to the Portuguese climatic and soil conditions. In a previous study conducted by Correia *et al.* (2018) it emerged as most relevant the differences between the climatic region of origin of the species and the current site. Therefore, we may suppose that exotic species that originate from similar climatic regions, would be better adapted to survive in the region. Despite of being exotic, some species might have equal or better survival rates than some of the native species. This would justify why *Q. shumardii*, native to southeast U.S.A., hence used to subtropical humid climates (Edwards, 1990), would have a high survival in Lisbon conditions. Similarly, *E. globulus* native from southeast Australia (Humphries *et al.*, 2005) is well adapted to temperate climate. Also *Q. petraea*, which is widely distributed in almost all of Europe, with exception of some Mediterranean locations (Humphries *et al.*, 2005) showed a high survival. Other exotic species well adapted to Mediterranean climates and which survived well in our conditions were *C. sempervirens* and *P. brutia*, both native to south Eastern Europe (Humphries *et al.*, 2005).

Additionally, survival rates did not differ in general between coniferous and broadleaved species. However, significant differences were found at species level within genus. Causes of mortality may further differ among species. Summer drought was a most important mortality factor for most species (Correia *et al.* 2018). We may also consider the existence of differences caused by other edaphoclimatic conditions, such as late spring frost which may cause the survival of broadleaved seedlings to diminish (Fisichelli *et al.*, 2014).

The fact of some species having a much smaller beginning number of individuals, could have further influenced the results due to random factors.

Damage

Lisbon and Sintra arboretums differed on damage intensity. In Lisbon arboretum had more pests and pathogens than Sintra. Which, in the end, might have contributed to Lisbon arboretum having more than 3 times the defoliation damage of Sintra. This difference might be justified by the fact that Sintra microclimate is characterized by lower temperatures and higher annual precipitation, which would not favour pests and pathogens to develop as quickly as in

Lisbon (Ratte, 1984; Hagstrum & Milliken, 1988). Also relevant is the fact that trees in Sintra have developed much faster and were considerably larger than in Lisbon.

Due to the edaphic-climatic conditions, trees in Sintra are most probably under low water stress than in Lisbon. It is well known that drought is one of the factors most favouring the occurrence of many pests and pathogens (Haile, 2000; Ayres & Lombardero, 2000; Grinnan *et al.*, 2013). Well-developed crown could have possibly a dilution effect on pests, which means that for a similar abundance of an insect pest the damage would be lower. Finally, taller trees in Sintra could have created observation difficulties, resulting in damage underestimation.

On overall, broadleaved species were more damaged than coniferous species. This could be due to a higher diversity and abundance of pest and pathogens more specialized in broadleaved species. This fact could also have happened due to differences between species in susceptibility to defoliators through defence mechanisms, such as volatile compounds (Paré & Tumlinson, 1999) and even through an asynchrony between them and the biotic agents.

Furthermore, it was observed a higher discolouration damage than defoliation, which lead us to believe that there is a higher number of abiotic stressors, and possibly pathogens, rather than pests. Regarding abiotic factors it is possible that the nutrient availability or climate conditions are not the best suited for most of the young trees from the studied genera. An opposite trend, with more defoliation than discolouration, was verified in two genera only *Quercus sp.* and *Fagus sp.* This fact may be justified by these particular genera having specific leaf shapes, as well as bigger and maybe more tender and nutritious leaves than the remaining and thus more attractive to defoliators (Rivero-Lynch *et al.*, 1996).

In general, defoliation, as observed in the Lisbon arboreta, was lower in exotic species when compared with the native congeneric species. Despite this, the differences were not homogeneous and not always significant. When discolouration was assessed, the same pattern was also verified, where exotic species also had a lower value than native species, 4.6% and 8.2%, respectively. Yet, variability was high and highly influenced by particular species. For example, discolouration among broadleaves was highly influenced by *C. siliqua* trees.

Regarding the most used species in Portuguese forestry, *P. pinaster* was the one with the lowest value both in defoliation and discolouration, where *Q. suber* had the highest defoliation value and *E. globulus* had the highest discolouration value.

Pines were additionally evaluated for pine processionary moth presence. This species is the most important defoliator of pines in the Mediterranean countries. Despite the Fisher's exact test showing that pine species are equally susceptible to the processionary moth, this was based only on three species which were affected, *P. pinaster*, *P. pinea* and *P. brutia*. This result may seem against our hypothesis and against previous studies showing that this

defoliator has pine species preferences (Jactel *et al.*, 2015). Yet, these three pine species are all also among the highest susceptible (Jactel *et al.*, 2015). The three species are also the most numerous in the arboreta, whereas the other species occur on rather small numbers, being not representative. Also, due to the arboretum young age, some pine trees are probably too small to be attacked by the processionary moth, since the females are attracted by taller trees silhouette (Régolini *et al.* 2014).

Seasonal variation on oaks trees

Damage observed along season on two oak species, a native *Q. robur* and the exotic oak species *Q. rubra*, showed a common increased trend accompanying leaf development, as expected. Still, in the end of the growing season, in June, the native species had four times more damaged leaves than the exotic one. This result agrees with the enemy release hypothesis, which predicts that outside its native range exotic species will be benefit by the lack of specialist enemies (Keane & Crawley, 2002). Nonetheless these results are contradictory with other studies where a high herbivory damage on exotic species was observed, mainly caused by generalist herbivores or herbivores from congeneric host tree species (Branco, *et al.*, 2015).

Additionally, from all the pests and pathogens that damaged the two oak species in Lisbon arboreta, we observed that the European oak, *Q. robur*, had a richer number of pests and pathogens than the American oak, *Q. rubra*. This observation agrees with the hypothesis that native species have a larger number of biotic agents, and therefore also contributing to a higher herbivory damage value in the end of the season.

A significant difference in phenology was further registered between the two species. The native species *Q. robur* had much earlier development than the exotic *Q. rubra*. This difference could be related to its origin. Originate from Northeast America, Sander (1990) shows that *Q. rubra* is adapted to withstand bitterly cold winters and semi-humid summers, which is also the climate from the provenances assessed for this species (temperate Atlantic).

We observed significant differences on bud burst timing and leaf development among provenances. We could divide six provenances in two different groups according to their phenology development (Table 13). Group 1 with earliest development was composed by individuals from *Q. robur* and whose provenances have a very similar climate to the Portuguese (QUROPAGO, from litoral vasco-navarro, QUROFRAN from France and QUROUNIT from New Forest, Hampshire). Group 2 with latest development was composed by the European oak provenance from Posavina, Croatia (QUROPOSA) and two other

provenances from *Q. rubra*. To note QUROPOSA is originate from a temperate continental climate while may justify its later development. Another provenance, QUROSUDO showed earlier development than the remaining provenances. But we could not add this provenance to the statistical analysis due to the existence of only one individual.

Our results showed that phenology performs a crucial role in herbivory damage, where provenances that had an earlier leaf development showed higher damage values and vice-versa. This result goes according to our hypothesis of herbivory being influenced by phenology, which was also shown in many studies (Collinge and Louda, 1989; Elzinga *et al.*, 2007; Mitchell Aide, 1992; Singer and Parmesan, 2010; Sukumar and Murali, 1993). In two particular provenances, QUROPAGO and QUROFRAN, differed from the remaining, with higher herbivory damage values and earlier development. The timing of bud bursting seems to be a determinant factor on this regard. Additionally, provenance was found relevant for different feeding guilds, namely for leaf-miners and skeletonizers, and rust and mildew, which may be active on different seasons.

5. Conclusion

In conclusion, climate change is altering ecosystem composition and with it the distribution of several tree species all around the world, as well as pests and pathogens. Infrastructures like the REINFFORCE are an important contribute to better understand what the future holds and for mankind to better cope with it.

The present study shows that phenology influences herbivory, as well as the existence of differences in herbivory damage caused when native species are compared to exotic species. We hope that this work may contribute to a better understanding of the current state of forest ecosystems and pests and pathogens. In the future, we think it would be beneficial if more studies regarding seedling/young trees survival from both native and exotic species were made as well as studies assessing the presence of new biotic agents and tree species.

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Annex

Annex 1. Number of individuals affected by each assessed damage type, in the ten analysed genera, from Lisbon arboretum.

Damage Type	<i>Acer</i>	<i>Betula</i>	<i>Castanea</i>	<i>Ceratonia</i>	<i>Cupressus</i>	<i>Eucalyptus</i>	<i>Fagus</i>	<i>Liquidambar</i>	<i>Pinus</i>	<i>Quercus</i>
9.1	1	16	2	14	0	4	1	1	9	256
9.2	0	0	0	0	0	3	0	0	0	18
9.3	0	0	0	1	0	0	0	0	0	65
9.4	1	1	0	0	0	0	0	0	0	73
9.5	0	0	0	0	0	0	0	0	0	27
9.6	2	7	1	31	0	5	0	1	10	121
9.7	0	0	0	0	0	0	0	0	2	1
9.8	0	0	0	0	0	0	0	0	1	1
6.1	0	1	0	0	0	0	0	0	0	39
6.2	0	1	0	0	0	0	0	0	0	38
6.3	3	15	2	38	0	12	1	1	0	120
6.4	0	0	0	0	3	0	0	0	83	0
6.5	0	0	0	0	0	0	0	0	2	0
6.6	0	0	0	0	1	0	0	0	70	0
3.1	0	0	0	0	0	0	0	0	1	0
3.2	0	0	0	0	0	0	0	0	0	1
3.4	0	0	0	2	1	0	0	0	25	4

Annex 2. Tukey post-hoc multiple comparisons test, regarding provenance phenology.

Multiple Comparisons						
Provenance (I)	Provenance (J)	Diff. (I-J)	Std. Error	Sig.	95% CI	
					Lower Bound	Upper Bound
FRAN	PAGO	0.889	2.477	0.999	-6.52	8.29
	POSA	-12.278*	2.961	0.002	-21.13	-3.43
	UNIT	-4.563	2.400	0.416	-11.74	2.61
	FEST	-20.778*	4.392	0.000	-33.90	-7.65
	VANA	-20.778*	3.376	0.000	-30.87	-10.69
PAGO	FRAN	-0.889	2.477	0.999	-8.29	6.52
	POSA	-13.167*	2.809	0.000	-21.56	-4.77
	UNIT	-5.452	2.210	0.158	-12.06	1.15
	FEST	-21.667*	4.291	0.000	-34.49	-8.84
	VANA	-21.667*	3.244	0.000	-31.36	-11.97
POSA	FRAN	12.278*	2.961	0.002	3.43	21.13
	PAGO	13.167*	2.809	0.000	4.77	21.56
	UNIT	7.714	2.741	0.075	-0.48	15.91
	FEST	-8.500	4.587	0.445	-22.21	5.21
	VANA	-8.500	3.626	0.200	-19.34	2.34
UNIT	FRAN	4.563	2.400	0.416	-2.61	11.74
	PAGO	5.452	2.210	0.158	-1.15	12.06
	POSA	-7.714	2.741	0.075	-15.91	0.48
	FEST	-16.214*	4.247	0.006	-28.91	-3.52
	VANA	-16.214*	3.185	0.000	-25.73	-6.69
FEST	FRAN	20.778*	4.392	0.000	7.65	33.90
	PAGO	21.667*	4.291	0.000	8.84	34.49
	POSA	8.500	4.587	0.445	-5.21	22.21
	UNIT	16.214*	4.247	0.006	3.52	28.91
	VANA	0.000	4.865	1.000	-14.54	14.54
VANA	FRAN	20.778*	3.376	0.000	10.69	30.87
	PAGO	21.667*	3.244	0.000	11.97	31.36
	POSA	8.500	3.626	0.200	-2.34	19.34
	UNIT	16.214*	3.185	0.000	6.69	25.73
	FEST	0.000	4.865	1.000	-14.54	14.54