Monitoring two REINFFORCE Network Arboreta: first result on site, climate and genetic interaction showing impact on phenology and biotic damages

Avaliando dois arboreta da rede REINFFORCE: primeiros resultados sobre interacção local, climática e genética, demonstrando o impacto sobre fenologia e danos bióticos

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Abstract

Understanding how climate affects tree phenology, biotic and abiotic vulnerability, is a most important research subject. Particularly, climate warming appears to lengthen the growing season, which may affect the exposition to insect pests and pathogens. Also, extreme weather events combined with shifts in phenology may have dramatic consequences, such as early leaf flushing exposure to freezing events. In this study 2 arboreta were followed in the south most distribution of the REINFFORCE Network (Lisbon and Sintra) for 3 species, one native (*Quercus robur* L.) and 2 non-native (*Quercus rubra* L. and *Betula pendula* Roth). Plant growth, leaf phenology and insect damage were assessed according to protocols defined under REINFFORCE project. Species x site interaction was found significant for survival and growth. Phenology differed between and within all species. No difference found for frost tolerance. *B. pendula* had earlier bud burst at both sites, resulting in a longer growth period, and higher stem growth, being least affected by insect damage, although survival was affected under higher temperature, where *Q. robur* performed better. This study corroborates the importance of REINFFORCE arboreta network as a tool to assess climate change impact on forest species and support assisted migration considering climate change scenarios.

Keywords: *Betula pendula*; *Quercus robur*; *Quercus rubra*; phenology; frost tolerance; pest damage

Resumo

Compreender como o clima afeta a fenologia das plantas e a sua vulnerabilidade biótica e abiótica é um assunto de extrema importância. Particularmente, quando o aumento da temperatura parece promover um alongamento do período de crescimento, o qual poderá afetar a exposição a pragas e agentes patogénicos. Adicionalmente, os eventos climáticos extremos combinados com as alterações na fenologia potenciam desfechos significantes, nomeadamente a antecipação da rebentação dos gomos com exposição a geada. Neste trabalho, foram monitorizadas 3 espécies, uma nativa (*Quercus robur* L.) e 2 não nativas (*Quercus rubra* L. and *Betula pendula* Roth), em 2 arboreta da rede REINFFORCE (Lisboa e Sintra). Foram avaliados o crescimento, fenologia foliar e danos por insetos, de acordo com os protocolos definidos sob o projecto REINFFORCE. A interacção Espécie x Site revelou-se significativa para sobrevivência e crescimento. Na fenologia observaram-se diferenças entre e dentro das espécies. Não foram observadas diferenças significativas para tolerância ao frio. A espécie *B. pendula* apresentou rebentação dos gomos mais precoce em ambos os locais, resultando num período de crescimento mais alargado e maior crescimento em altura, sendo menos afetada por danos por insetos, embora a sobrevivência tenha revelado suscetibilidade a temperatura mais elevada, onde a espécie *Q. robur* apresentou melhor performance. Este estudo corrobora a importância da rede de arboreta REINFFORCE como ferramenta para monitorizar o impacto das alterações climáticas nas espécies florestais e proporcionar bases para a migração assistida de espécies, considerando os cenários de alterações climáticas.

Palavras-chave: *Betula pendula*; *Quercus robur*; *Quercus rubra*; fenologia, tolerância ao frio; danos por pragas

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INTRODUCTION

Climate change is now accepted as one of the most important phenomenon affecting the future of the world's natural systems and, in turn, human society. Many published reports have presented significant evidence that climate changes over the past fifty years have affected several aspects of forest ecosystems. These include tree growth and dieback, invasive species problems, species distributions and migrations, seasonal patterns in ecosystem processes, demographics and even extinctions (IPCC, 2007a). Future climate scenarios in the Mediterranean region indicates a systematic 3 to 4 °C increase in average temperature and reduction in annual rain fall by 20 to 40% (IPCC, 2013). Consequently, in this region, species distribution may be mainly driven by the stress caused by the increase of arid and semi-arid regions (SANTOS et al., 2002). The combined outcome of droughts and high temperatures will cause further lower carbon sequestration in some areas.

Forests also influence local climate (ELLISON, 2017), and thus afforestation programs may play a decisive role on climate regulation at both local and global levels. At the same time, due to climate change, trees are facing physiological stress, variations in phenology, and variations in the exposition to pests and diseases. Therefore, mutual interaction between forests and climate change has become a most important research issue. REINFFORCE project established a network of arboreta with the aim to improve our general understanding of species capacity to cope with climate changes. The same genetic materials was planted under 38 different climates, from the south of Portugal to the south of Scotland (along a range of latitudes from 37° to 58°) covering the European Atlantic temperate forests distribution. Programs like REINFFORCE create physical infrastructures to study adaptation of forest trees to climate change (ORAZIO, 2009, 2013).

Many forest ecosystems studies have correlated recent climate trends with changes in phenology as well as with changes in forest productivity (ROSENZWEIG, 2007). Such studies have indicated that climate warming appears to lengthen the growing season and increase tree growth rates in many boreal and temperate forests. However, for Mediterranean regions, studies suggested that the warming has contributed to measurable reductions in forest productivity through interactions with drought, fire and biotic disturbance (ROSENZWEIG, 2007). From our literature review there are few studies addressing the effect of climate on the tree phenology in Mediterranean climates and its impact on biotic and abiotic risks. For temperate regions, it was demonstrated that changes in phenology can affect ecological relationships, for example, by creating a mismatch between plant flowering time and the presence of insect pollinators (POST; FORCHHAMMER, 2007; ROSENZWEIG, 2007). On the other hand, as many herbivorous insect species can only develop on young plant material, there could be a mismatch between plant phenology and active larval stages. It may also occur on some insect species, that changes in the phenology of leaves growth or growing tips can cause shifts in the phenology of herbivorous insects larval development (VISSER; HOLLEMAN, 2001). Intra-specific variability in plant phenology may further affects the window of exposition to herbivore insects (SAMPAIO et al., 2016).

In addition, changes in climate variables may have direct influence on insect pests and pathogens affecting both their survival and development. There is further evidence that warmer temperature is generally favorable for insects by shortening their life cycle (CURRANO et al., 2008; FRAZIER; HUEY; BERRIGAN, 2006), although extreme hot temperature may also cause important insect mortality dependent on life stage and its phenology (e.g. SANTOS et al., 2011). It also can have indirect influence brought by the interactions between host species and their herbivores (AYRES; LOMBARDERO, 2000).

Climate change indicators includes not only shifts in mean conditions but also changes in the frequency and timing of extreme weather events such as severe cold, spring frost and extended drought (SOLOMON, 2007; MARINO et al., 2011). Consequently, tree seedlings are responding to pressures of both mean, and extreme conditions. For example, mean temperature and the occurrence of late spring frost may strongly affect the emergence, development, growth, and survival of plants (FISICHELLI; VOR; AMMER, 2014; SANTOS et al., 2002). Specifically, and as a by-product of warmer temperatures, the occurrence of frost after leaf total flushing is projected to become more common phenomenon in some parts of the world (MEEHL, 2000, GU; HANSON; POST, 2008). This scenario has a number of important ecological implications. In particular, the newly developed leaves are sensitive to frost events as they lack the structural rigor necessary to prevent damage. Depending on the timing of spring warmth, early and accelerated leaf development has the potential to increase the frequency and magnitude of leaf damage from freezing events (INOUE, 2008). Resultant lasting effects include the loss of stored carbon and nutrients as well as reduced photosynthetic carbon gain

(DAWES et al., 2010; GU; HANSON; POST, 2008), and utterly, increase mortality of young trees. Additional studies show that plant grown under elevated CO₂ present decreased freeze tolerance, making even the most freeze-tolerant species more vulnerable to potential frost damage at warmer freezing temperatures. The most responsive species to these are likely to occur in the cool to cold climates at high latitudes and altitudes where seasonal temperatures and the length of frost-free period are important determinants of the growing season (CHEN; BURKE; GUSTA, 1995). A freezing event will thus injure these freeze-tolerant plant species depending on the acclimation state of the plant which is also affected by elevated CO₂ levels (LOVEYS; EGERTON; BALL, 2006).

This study aimed to analyze budburst in one locally native (*Quercus robur* L.) and two non-native species (*Quercus rubra* L., *Betula pendula* Roth) at two of the REINFORCE arboreta, and characterize they're survival, growth, physiological, pest attack and leaf phenology, in response to the climatic conditions on test sites. Each species is represented by contrasting provenances (in order to sample species' variability), since adaptive traits are related to geographic origin. This knowledge contributes for the selection of forest reproductive material to be used in afforestation programs, as well as support assisted migration to mitigate climate change effects.

MATERIALS AND METHODS

Field description

The study took place at two arboreta: Lisbon (Lat: 38° 42' 51.07" N; Long: 9° 11' 31.66" W; altitude: 106 m) and Sintra (Lat: 38° 46' 48.29" N; Long: -9° 24' 48.92" W; altitude: 400 m). Lisbon arboretum was installed in spring 2012, and Sintra arboretum in winter 2012.

Sites differ on soil, climatic and meteorological characteristics observed during the period following arboreta establishment (Appendix 1; ORAZIO, 2013). Sintra is characterized by higher humidity and a fresher summer in comparison to Lisbon. Considering the difference between climate conditions observed at each arboreta and at provenance sites, observations revealed consistently higher temperature differential at Lisbon for the summer period, and at Sintra all year round, with particular higher differential for the winter period (Fig. 1). There was also higher precipitation deficit at Lisbon, and winter excess at Sintra.

For this study, three broad leaves species were chosen, *Q. robur* (QURO), *Q. rubra* (QURU), *B. pendula* (BEPE). QURO is the only native to the studied area; QURU is native to North America. BEPE is mostly distributed in the Center and North of Europe, absent from the Iberian Peninsula except for some restricted high mountain areas (VAKKARI, 2009). QURO was represented by four provenances (FRAN from France; UNIT from UK; PAGO from Spain; POSA from Italy), QURU by two (FEST from France; VANA from Spain), and BEPE by three (NORD from France; KRAL from Krakova Slovakia; UNIT from UK). Provenances' detailed information is provided in appendix 2. At least twelve seedlings were observed per provenance. For QURO and BEPE, 36 plants per provenance were installed. For QURU, 12 plants per provenance were installed. All of these plants were included in the study.

Meteorology

Temperature and precipitation were monitored hourly by an automatic local weather station placed at each arboreta.

Meteorological variables difference was calculated between arboreta observed data (2012 - 2014) and provenance site data (1961 - 1990) obtained from ClimateEU v4.63 software package, available at <http://tinyurl.com/ClimateEU>, based on methodology described by Hamann et al. (2013)

Plant survival

Survival was evaluated in May 2014. Survival is expressed as the percentage of the living seedling recorded since plantation to 2014, for each provenance.

Growth measurement

Growth measurements were carried out in November 2013. Height (cm) was measured for the tallest living plant branch/bud using extensive pole, with 1 mm precision. Diameter (mm) was measured at 2 cm from ground with a digital caliper, with 0.01 mm precision, in two crossed measurements. Growth is determined as the difference between initial measurement, at installation, and 2013 measurement.

Note: provenance climate data (1961 – 1990) obtained from ClimateEU v4.63 software package, available at <http://tinyurl.com/ClimateEU>, based on methodology described by Hamann et al. (2013)

Figure 1: Meteorological variables difference between *arboreta* (2012 - 2014 data) and provenance site (1961 - 1990): a) Mean maximum temperature; b) Mean minimum temperature; c) Precipitation. BEPE- *Betula pendula*; QURO- *Quercus robur*; QURU- *Quercus rubra*. Provenance designation and information are described on appendix 2 and ORAZIO (2013).

Figura 1: Diferença entre as variáveis climáticas dos *arboreta* (2012-2014) e locais de proveniência (1961-1990): a) Média das temperaturas máximas; b) média das temperaturas mínimas; c) Precipitação. BEPE- *Betula pendula*; QURO- *Quercus robur*; QURU- *Quercus rubra*. As designações e informações sobre as proveniências de cada espécie estão descritas no apêndice 2 e em ORAZIO (2013).

Phenology

At Lisbon, phenological status was evaluated weekly, from the last week of February 2014, until complete budburst. The best branch with highest numbers of buds in each direction (west, east, north and south) was chosen and tagged for follow-up. The buds in the tagged branches were closely observed every week to check the stage of development, following the phenology protocol defined under REINFFORCE project (reinforce.iefc.net).

On the 3rd and 4th of March, the phenology observation was carried out at both Lisbon and Sintra, to compare phenology status at this specific time.

Insect damage

At the mid of April, when the leaves of all the seedlings have expanded, one field observation was done to monitor insect damage on the three species and all the 10 provenances. For that, the protocol of biotic damage defined under REINFFORCE project (reinforce.iefc.net) was applied. Damage and severity, as expressed by the proportion of attacked leaves per branch, were registered.

Spring frost tolerance

Frost tolerance was evaluated for two species (*QURO* and *BEPE*) in the end of May, through cell membrane injury in leaf discs measuring electrolyte leakage conductivity, after artificial freezing. Each species was represented by three provenances. Seven seedlings from each provenance were sampled. Five fully expanded leaves from each seedling were collected, and a composite sample with one leaf disc per each seedling and per provenance was prepared in a vial tube. Three tubes per provenance and per species were placed in each freezing bath. Frost treatment was induced in a cryostat (Aralab, Lisbon, Portugal) with three baths containing an aqueous ethylene glycol solution. A controlled freezing program followed a constant cooling and thawing rate of 4 °C/ h and 2 h exposure to five target freezing temperatures (–3, –5, –6.6, –9, –10.5 °C). When the temperature of the bath was at –2 °C, about 0.5 g of finely crushed ice (from deionized water) was added to each tube to make contact with the leaf discs avoiding super cooling. Bath temperature was monitored via thermocouples sensors connected to a data logger (DL2, Delta-T). After the freezing treatment, 15 ml of deionized water was added to each tube, the tubes were then kept for 24 hours at water bath at 25 °C. Electrolyte conductivity was then measured in each tube (T1) with a K220 conductivity meter (Consort, Turnhout, Belgium). The samples were boiled in an autoclave at 120 °C for 10 min and held at 25 °C for 24 h before measurement of maximum electrolyte conductivity (T2). Relative injury (RI) was expressed as a ratio of electrolyte conductivity measured after freezing treatment to maximum electrolyte conductivity, $RI = (T1/T2) * 100$ (COSTA E SILVA et al., 2009; ROCHA et al., 2013). RI was then used to calculate TL50 for each provenance (which is an assessment of the temperature that causes death to 50% of plants).

Statistical analysis

A generalized linear model was adjusted for survival, proportion of attacked leaves (insect damage), bud burst percentage, frost damage percentage, using a logit link, and height and diameter growth. ANOVA procedure was used to assess provenance (nested within species), species and site effect significance for height growth, diameter growth, number of days (since January 1st) until bud burst occurred (defined by reaching the stage number 10) and frost damage variables, in order to assess the effect of species and provenances. Model normality assumptions were verified through graphical analysis and Levene test. Wald Chi-square test was used for model significance. Fisher's least significant difference (LSD) was used for post-hoc multiple comparisons. Species effect on phenology was tested through Kruskal-Wallis, and comparison between species with Mann-Whitney.

The analysis of phenological stages, bud burst, insect damage, and frost damage was done using SPSS software (ver. 22.0). Analysis of survival and growth was performed with R (ver. 3.3.2) software.

Graphics were generated with visreg package for R.

RESULTS

Survival

Survival was higher at Sintra (Fig 2). Interaction site x species was found to be significant (table 1 in appendix 3). For the two non-native species, survival was much higher at Sintra than Lisbon, whereas for *QURO* differences in survival between the two sites were not so high (Fig. 2). At Sintra, survival was highest for *BEPE*, whereas at Lisbon, survival was highest for *QURO*.

Growth

Tree growth was higher in Sintra, regarding both height ($F = 29.465$, $P < 0.001$) and diameter ($F = 97.318$, $P < 0.001$). A significant *species: site* interaction was observed for height and diameter (Figure 3 in appendix 4).

No significant difference was found between provenances for height growth within species– either at Lisbon or Sintra (S4). For *BEPE*, *provenance* is almost significant ($P = 0.08$), with evidence that KRAL and NORD provenances have higher height growth values for Sintra (appendix 5).

As for diameter growth, *provenance* is significant for *QURU* (appendix 4), and FEST has significantly higher growth than VANA at Sintra (appendix 5, Figure 4).

Note: Horizontal line represents estimated probability. Grey bars represent 95% confidence interval.

Figure 2: Estimated provenance/species survival probability at Sintra and Lisbon for 2-year plantation. a) *Quercus rubra*, b) *Quercus robur*, c) *Betula pendula*, d) All species. BEPE- *Betula pendula*; QURO- *Quercus robur*; QURU- *Quercus rubra*. Provenance designation and information are described on appendix 2 and ORAZIO (2013).

Figura 2: Probabilidade de sobrevivência estimada para as espécies/proveniências em Sintra e Lisboa, para plantações com 2 anos. a) *Quercus rubra*, b) *Quercus robur*, c) *Betula pendula*, d) todas as espécies. BEPE- *Betula pendula*; QURO- *Quercus robur*; QURU- *Quercus rubra*. As designações e informações sobre as proveniências de cada espécie estão descritas no apêndice 2 e em ORAZIO (2013).

Table 1. Analysis of deviance table for GLM (type III test) for survival.

Table 1. Resultados da análise de "desvios" para os modelos lineares generalizados (teste do tipo III) em relação à sobrevivência.

Species	Variance source	Chisq	Df	Pval
BEPE	provenance	0.599	2	
	Site	297.560	1	***
QURO	provenance: site	2.658	2	
	provenance	5.5128	3	
QURU	Site	24.3180	1	***
	provenance: site	1.2115	3	
All	provenance	0.2022	1	
	Site	1.5272	1	
	provenance: site	6.6433	1	**
	species	48.66	2	***
	Site	330.41	1	***
	species: site	96.91	2	***

Nota: Pval código de significância ("***" < 0.0001 < "**" < 0.001 < "*" < 0.05 < "." < 0.1 < " " < 1)

Note: Horizontal line represents mean and grey bars represent standard error.

Figure 3: Provenance/species mean height growth at Sintra and Lisbon for 2-year plantation. a) *Quercus rubra*, b) *Quercus robur*, c) *Betula pendula*, d) All species. BEPE- *Betula pendula*; QURO- *Quercus robur*; QURU- *Quercus rubra*. Provenance designation and information are described on appendix 2 and ORAZIO (2013).

Figura 3: Crescimento médio em altura em Sintra e Lisboa para uma plantação com 2 anos. a) *Quercus rubra*, b) *Quercus robur*, c) *Betula pendula*, d) todas as espécies. BEPE- *Betula pendula*; QURO- *Quercus robur*; QURU- *Quercus rubra*. As designações e informações sobre as proveniências de cada espécie estão descritas no apêndice 2 e em ORAZIO (2013).

Phenology

The three studied species significantly differed on the budburst date ($F_{2,81} = 18.22$, $P < 0.001$). Kruskal-Wallis test also showed significant differences between species on the phenology stage observed in Lisbon arboreta during the period of three months since the first of March till the end of May ($Z = 72.64$, d.f. = 2, $P < 0.001$). All BEPE's provenances have an early bursting comparing with the other species, while the two provenances of QURU showed the latest bursting (Fig 5). Further significant difference were found among the four provenances of QURO ($F_{3,54} = 3.56$, $P = 0.024$), while the provenances of the other species showed no significant differences: QURU ($F_{1,5} = 0.042$, $P = 0.839$), BEPE ($F_{2,26} = 1.81$, $P = 0.183$).

Comparing bud bursting for each of the provenance between Lisbon and Sintra, six provenances, BEPE Nord, BEPE Unit, QURO Pago, QURO Posa, QURO Unit and QURU Vanna, were earlier to burst at Sintra. Two provenances QURO Fran and QURU Fest, showed similar bud burst timing in both locations. Only BEPE Kral was earlier to sprout in Lisbon than Sintra (Fig. 5). Nevertheless, when applying Mann-Whitney test to compare the phenology stage in both sites at (3 and 4 March, 2014) no significant difference was found ($Z = -1.044$, d.f. = 1, $P = 0.296$).

Note: Horizontal line represents mean and grey bars represent standard error.

Figure 4: Provenance/Species mean diameter growth at Sintra and Lisbon for 2-year plantation. a) *Quercus rubra*, b) *Quercus robur*, c) *Betula pendula*, d) All species. BEPE- *Betula pendula*; QURO- *Quercus robur*; QURU- *Quercus rubra*. Provenance designation and information are described on appendix 2 and ORAZIO (2013).

Figura 4: Crescimento médio em diâmetro (colo) para as espécies/proveniências em Sintra e Lisbon para uma plantação com 2 anos. a) *Quercus rubra*, b) *Quercus robur*, c) *Betula pendula*, d) todas as espécies. BEPE- *Betula pendula*; QURO- *Quercus robur*; QURU- *Quercus rubra*. As designações e informações sobre as proveniências de cada espécie estão descritas no apêndice 2 e em ORAZIO (2013).

Insect damage

The main damage type observed consisted on chewed leaves (86 to 99% among all types of damage). Leaf miners, sap feeders, galls and sketizers showed very low level of occurrence. The probability of suffering leaf damage was overall highest for *QURO* leaves at Sintra (0.49) (Table 2). At Lisbon, *QURU* had similar values of leaf damage as *QURO*, whereas in Sintra values were slightly but significantly lower in comparison with the congeneric native tree species (Tables 4). *BEPE* on the other hand showed the lowest probability of damage in both sites (0.09 and 0.18 at Lisbon and Sintra, respectively) (Table 2).

The comparison between the native species (*QURO*) with the average of the non-native species also revealed a significant difference on insect damage for both Lisbon ($F_{1,82} = 0.018$, $P < 0.001$), and Sintra ($F_{1,185} = 0.013$, $P < 0.001$).

The provenances of *BEPE* in Lisbon did not differ on the insect damage ($Z = 2.25$, d.f. = 1, $P = 0.13$), nor did the provenances of *QURO* ($Z = 3.61$, d.f. = 1, $P = 0.057$), or *QURU* ($Z = 0$, d.f. = 1, $P = 1.0$). Yet, for Sintra, provenances of *QURO* differed significantly ($Z = 4.32$, d.f. = 1, $P = 0.037$). Still, there were no significant differences observed among the provenances of *BEPE* and *QURU* ($Z = 2.58$, d.f. = 1, $P = 0.1$) ($Z = 3$, d.f. = 1, $P = 0.83$).

Figure 5: Evolution of budburst (percentage of total observed plants) per species and provenance along observation period, at Lisbon arboretum. BEPE- *Betula pendula*; QURO- *Quercus robur*; QURU- *Quercus rubra*. Provenance designation and information are described on appendix 2 and ORAZIO (2013).

Figura 5: Evolução da rebentação dos gomos(percentagem do total de plantas observadas) por espécie e proveniência, durante o período de avaliação, no arboretum de Lisboa. BEPE- *Betula pendula*; QURO- *Quercus robur*; QURU- *Quercus rubra*. As designações e informações sobre as proveniências de cada espécie estão descritas no apêndice 2 e em ORAZIO (2013).

Table 2. Percentage of damaged leaves (average $\pm$ standard error) at Lisbon and Sintra for each tree species.

Table 2. Percentagem de folhas apresentando danos (média $\pm$ erro padrão) para os arboreta de Lisboa e Sintra, para cada espécie.

Species	Lisbon	Sintra
<i>Betula pendula</i>	9 $\pm$ 1.2 ^a	18 $\pm$ 0.7 ^a
<i>Quercus robur</i>	40 $\pm$ 1.3 ^b	49 $\pm$ 1.4 ^c
<i>Quercus rubra</i>	40 $\pm$ 4.8 ^b	31 $\pm$ 2.7 ^b

Note: Values followed by the same letter per site do not differ significantly ($\alpha = 0.01$).

Spring frost tolerance

QURO and BEPE showed similar RI when subjected to negative temperatures ranging from -3 to -10.5 °C (41.39 $\pm$ 1.73 vs. 39.05 $\pm$ 1.87, respectively). Calculated TL50 (appendix 6) was also similar between QURO (-7,18 $\pm$ 0,025 °C) and BEPE (-7,05 $\pm$ 0,067 °C).

No statistical effect found for provenance on frost damage, both for QURO ($F_{2,6} = 0.241$, $P = 0.787$) and BEPE ($F_{2,6} = 1.19$, $P = 0.317$). Fitting a generalized linear model for frost damage (with provenance nested in species effect), for TL50 -6.6 °C temperature, no significant effect was found between species ($Z = 0.42$, d.f. = 1, $P = 0.51$).

DISCUSSION

Although the REINFFORCE network's full range is composed by 38 sites, this preliminary work aims to analyze a small window of locations. The two study sites are considered of particular interest, as they are the most southern located within the REINFFORCE arboreta range, characterized mainly by higher temperatures and dryer climate which may dramatically affect tree survival, growth and risk to abiotic and biotic factors.

Meteorological conditions differed between sites, maximum summer temperatures were lower and air humidity higher at Sintra in comparison with Lisbon. As soil type also differs, soil water extraction by plants at Lisbon arboretum (Loam-Sandy clay) would be more difficult, since soil matric potential is expected to be considerably lower. This could have had a major impact on plant survival and growth.

Survival and growth

The *site*, *provenance: site* and *species: site* significant effect, show how some species/provenance respond differently at each site, highlighting the relevance of the edaphoclimatic conditions, especially for survival. Sintra *arboretum* seems to be more favorable to the overall performance of all species. At Sintra, *BEPE* outperforms the other species in every aspect, with *NORD* and *UNIT* provenances standing out at height growth. Even at Lisbon, this species has a higher height increment than the others, although survival is compromised. Interestingly, *QURO* presented the higher survival rate at Lisbon, without having any superior effect on growth for that arboreta. *QURU* had the lowest survival at Lisbon, but does not differ in growth from the previous. *QURO* is known to have higher drought tolerance than *BEPE* (VAN HEES, 1997), sustaining it even under higher temperature (KUSTER et al., 2012), which helps explain its advantage at the lowest survival site (Lisbon) (Fig. 2, 3 and 4), as plants develop higher root growth under water stress, than under water availability (OSONUBI, 1981). For *BEPE*, drought impact is amplified by an increase in summer air temperature (KHARUK et al., 2013), so this corroborates for the higher mortality and lower growth rate observed at Lisbon *arboretum*. *BEPE*'s earliness of budburst contributes to its high annual growth rate (ZAPATER et al., 2012) as observed in our results, typical for pioneer species like this one (HYNENEN, 2008). Drought can trigger an early investment in deep root growth resulting in slower above ground growth, so this explains the reduced diameter growth observed at Lisbon arboretum, according to Kuster (2012). There is also an observed difference for diameter growth in *QURO* between sites, although height growth was similar. In Kuster (2012), species presented a lower diameter to height growth ratio under high temperatures, varying greatly with provenance. So the assumption that the significant lower diameter growth observed for *FRAN* and *POSA* (Table 6) can be a direct influence of higher temperature and precipitation summer deficit from provenance conditions (Fig.1).

For *QURU*, growth increase also corresponds to lower differences for temperature and precipitation for the early growing season (May through July) as also found by LeBlanc and Terrell (2011), and that under increasing water stress more growth is allocated to roots than shoots (JACOBS, 2009). So, *FEST* provenance shows higher growth at Sintra, and *VANA* the highest at Sintra.

Phenology

The strong relationship that has been found by Fu (2012) and Pletsers (2015) between winter temperature and dormancy release implies that even small changes in winter temperature can have large impacts on the timing of bud burst. As a reaction to global warming, the chilling requirement might not be fully met, and thus bud burst could then be delayed (KONNERT et al., 2015). Alternatively, in an environment where the chilling requirement is presently far exceeded, bud burst could occur earlier than at present, especially for early flushing species, under the influence of warmer winter and spring temperatures (FU, 2012). When comparing the results of our bud burst observations on March 2014, at Lisbon and Sintra there were no significant differences between the two sites which might be justified by similar winter temperatures (but not summer temperature) (Fig.1), latitude and thus photoperiod is also similar as the two sites are near to each other. Yet, on each site there are clear differences among species, with earlier budburst for *BEPE* and among *Q. robur* provenances, which can be strongly related to provenance altitude, according to Alberto (2011). *Quercus rubra* is considered to be a late species for bud burst and flowering time in its native range (VIEITEZ, 2012), and our study confirms that the species keep this late bud burst pattern both in Lisbon and Sintra.

4.3. Insect damage

Insect damage tended to be higher for the native *QURO*, with overall significantly higher values of leaf damage than the non-native species. This could be justified by the fact that outside its native range plants will be benefit of a release from their native natural enemies (KEANE, 2002). Nevertheless, *QURU* also had higher levels of damage by herbivores even though it is a non-native species in the

area. This result is justified due to the fact that this oak species' share close phylogenetic relationship with *QURO*, and thus is expected to have similar physical and chemical characteristics (ALI; Agrawal, 2012). Indeed, it is expected that the herbivores which feed on *QURO*, may also expand its host range to feed on the non-native oaks species *QURU* (BRANCO et al., 2015).

In contrast, *BEPE* was the one with lower insect damage in both sites. Unlike *Quercus* sp, *BEPE* does not have any phylogenetic close tree species (*i.e.* congeneric) native in the area of study, which therefore would reduce the hypothesis of host shifts (BRANCO et al., 2015). In alternative, from our results, *BEPE* was the first species to budburst, in March, and it is possible to hypothesize that bud burst may have occurred in a period when the activity of chewing insects was still low. If the leaves of *BEPE* were already matured when the feeding activity of insects was higher, then the leaf toughness and the amount of defensive compounds of these mature leaves could make them less edible for the insects (VAN ASCH, 2007). In fact, it is considered that early budburst might be a way of plants to escape herbivores (BOTH, 2009).

4.4. Spring frost tolerance

A number of relevant cold studies showed that *BEPE* and *QURO* have high cold tolerance during winter, and that the minimum lowest temperature for *QURO* can be as low as -40 and -35 for *BEPE* (MALIOUCHENKO, 2007; OLALDE, 2002). However, the purpose behind doing this test at the end of May was to simulate the occurrence of late spring frost, and to test the hypothesis that the seedling will be very sensitive to frost, taking into consideration that the leaves were still new, thin and not harden yet.

The performed test showed that both species were highly tolerant to cold, and the chosen provenances within the species were not a significant factor in these results. Still, *BEPE* was a little more sensitive to frost than *QURO* which also confirm other relevant studies concerning the species tolerance to frost during the coldest month of the year (MALIOUCHENKO, 2007; OLALDE, 2002).

CONCLUSION

Although this study relies on very early stage results at two REINFFORCE arboreta, limiting the number of plants available for sampling, it is still considered as a starting point for further studies covering the Arboreta Network, allowing simultaneously 38 environments and an extended sampling for species and provenances.

Studies performed in natural conditions can be harder to control, with some probability to be affected by unexpected factors impacting the vegetable material beside the ones that are in direct study. Exactly for that, they are of extreme importance, allowing to test multiple impacts of those diverse factors on the material, even for the unexpected ones.

In the present study, plant growth and survival responded to temperature and precipitation differences. Main findings point out that climate constrains like high temperature and low water availability will impact tree species in the early stage of tree plantation in multiple ways. It may have a direct impact on growth, shortening the growth period, and lowering initial biomass productivity, highly important for an efficient plant establishment. Nevertheless, this effect can be mitigated with the use of early budburst species, initiating growth in a more favorable period.

Non-native tree species might provide escape to herbivore insects but only if phylogenetically distant from native ones. Provenances generally behaved differently at each *arboreta*, stressing the need for selection of material adjusted for each present/future condition.

In general, it is yet too early to extract finer results, considering that the arboreta network needs a long period of time to provide a conclusive result. Further studies will continue this research and deeper this subject by studying more species of the arboreta, under the full range of the network

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Appendix 1. Characteristics of the study sites. Climatic variables correspond to the mean of the 1971-2000 periods (Source: IPMA)

Apêndice 1. Características dos locais de estudo. As variáveis climáticas correspondem à média dos períodos de 1971-2000 (Fonte: IPMA)

site variables	Lisbon	Sintra
altitude (m)	106	400
Climatic classification (Köppen)	Mediterranean Csa	Mediterranean Csb
Soil type	loam-Sandy clay loam	sandy-loam
Ph	6.4	4.3
maximum temperature (°C)	20.0	18.2
minimum temperature (°C)	12.5	12.3
mean precipitation (mm)	725	786
mean temperature (°C)	14.9	13.9

Appendix 2. The distribution of the provenances for the studied species in the arboreta. Climate data period 1961-1990 generated with the ClimateEU v4.63 software package, available at <http://tinyurl.com/ClimateEU>, based on methodology described by Hamann et al. (2013)

Apêndice 2. Distribuição das procedências das espécies estudadas na arboreta. Período de dados climáticos 1961-1990 gerado com o pacote de software ClimateEU v4.63, disponível em <http://tinyurl.com/ClimateEU>, baseado na metodologia descrita por Hamann et al. (2013)

Species	provenances identification - Codes	latitude	longitude	altitude (m)	mean temperature °C	annual precipitation mm
<i>Q. rubra</i>	Auberive, France - FEST	47.79	5.10	400	9.2	972
	Litoral Vasco, Spain -VANA	43.30	-2.03	210	13.5	1338
<i>Q. robur</i>	Ruisseau de Tiolet, France- FRAN	46.21	2.20	380	10.1	828
	New forest, Hampshire-UK - UNIT	43.2	-2.43	140	13.8	1192
	Navarro-Spain-Pago	50.85	-1.62	45	10	782
	Posavina-Croatia -POSA	44.47	16.46	1200	5.6	1184
<i>Betula</i>	Nord Est et Montagne France -NORD	48.39	5.98	470	8.2	918
<i>pendula</i>	Kralova-Slovakia -KRAL	48.352	17.32	240	9	697
	Wales-UK -UNIT	52.405	-4.03	15	9.5	1112

Appendix 3. Survival multiple comparison test for interaction using Wald Chi-square statistic. *Pval* significance code ("****" < 0.0001 < "***" < 0.001 < "**" < 0.05 < "." < 0.1 < " " < 1).

Apêndice 3. Teste de comparação múltipla de sobrevivência para interação usando estatística de Qui-quadrado de Wald. Código de significância *Pval* ("****" < 0,0001 < "***" < 0,001 < "**" < 0,05 < "." < 0,1 < " " < 1).

Species	Fixed	Pairwise	Value	Df	Chisq	Pval
BEPE	KRAL	Lisbon-Sintra	0.044776	1	77.306	***
	NORD	Lisbon-Sintra	0.019802	1	83.675	***
	UNIT	Lisbon-Sintra	0.042254	1	33.759	***
	Lisbon	KRAL-NORD	0.60000	1	2.0710	
	Lisbon	KRAL-UNIT	0.50000	1	0.0000	
	Lisbon	NORD-UNIT	0.40000	1	1.0503	
	Sintra	KRAL-NORD	0.39264	1	0.8506	
	Sintra	KRAL-UNIT	0.48485	1	0.0135	
	Sintra	NORD-UNIT	0.59281	1	0.4494	
QURO	Residuals			209		
	FRAN	Lisbon-Sintra	0.27778	1	3.6583	.
	PAGO	Lisbon-Sintra	0.23077	1	9.4229	**
	POSA	Lisbon-Sintra	0.25000	1	7.9000	*
	UNIT	Lisbon-Sintra	0.33333	1	4.7565	.
	Lisbon	FRAN-PAGO	0.44444	1	0.2227	
	Lisbon	FRAN-POSA	0.50000	1	0.0000	
	Lisbon	FRAN-UNIT	0.50000	1	0.0000	
	Lisbon	PAGO-POSA	0.55556	1	0.2227	
	Lisbon	PAGO-UNIT	0.55556	1	0.2963	
	Lisbon	POSA-UNIT	0.50000	1	0.0000	
	Sintra	FRAN-PAGO	0.38424	1	1.2370	
	Sintra	FRAN-POSA	0.46429	1	0.1137	
	Sintra	FRAN-UNIT	0.56522	1	0.3743	
	Sintra	PAGO-POSA	0.58140	1	1.3002	
	Sintra	PAGO-UNIT	0.67568	1	6.2073	
	Sintra	POSA-UNIT	0.60000	1	1.8870	
	Residuals			268		
QURU	FEST	Lisbon-Sintra	0.26316	1	1.4768	
	VANA	Lisbon-Sintra	0.00000	1	0.0001	
	Lisbon	FEST-VANA	0.6	1	0.2006	
	Sintra	FEST-VANA	0.0	1	0.0001	
All	Residuals			44		
	BEPE	Lisbon-Sintra	0.034428	1	207.777	***
	QURO	Lisbon-Sintra	0.266504	1	63.120	***
	QURU	Lisbon-Sintra	0.097765	1	97.859	***
	Lisbon	BEPE-QURO	0.29939	1	35.2023	***
	Lisbon	BEPE-QURU	0.52208	1	0.2271	
	Lisbon	QURO-QURU	0.71882	1	28.4535	***
	Sintra	BEPE-QURO	0.81324	1	43.9947	***
	Sintra	BEPE-QURU	0.76851	1	20.6962	***
	Sintra	QURO-QURU	0.43259	1	2.0588	
	Residuals			533		

Appendix 4. Analysis of Variance table (type III test) for growth. *Pval* significance code ("****" < 0.0001 < "***" < 0.001 < "**" < 0.05 < "." < 0.1 < " " < 1).

Apêndice 4. Análise da tabela de variância (teste do tipo III) para crescimento. Código de significância *Pval* ("****" < 0,0001 < "***" < 0,001 < "**" < 0,05 < "." < 0,1 < " " < 1).

Species	Variance source	Height			Diameter		
		SS	Df	F(Pval)	SS	Df	F(Pval)
BEPE	provenance	567.9	2	2.781(.)	7.58	2	0.352
	Site	2515.4	1	24.641(***)	631.21	1	58.656(***)
	provenance:Site	334.4	2	1.638	5.76	2	0.268
	Residuals	9800.0	96		1194.50	111	
QURO	provenance	82.9	3	0.410	42.32	3	2.066
	Site	49.8	1	0.739	114.97	1	16.836(***)
	provenance:Site	314.2	3	1.554	43.47	3	2.122
	Residuals	7481.9	111		976.58	143	
QURU	provenance	44.98	1	1.279	50.781	1	15.121(**)
	Site	86.48	1	2.458	18.070	1	5.381(*)
	provenance:Site	21.03	1	0.5977	8.020	1	2.388
	Residuals	633.27	18		60.448	18	
All	Species	561.4	2	12.079(***)	9.80	2	1.795
	Site	718.7	1	30.924(***)	156.78	1	57.429(***)
	Species:Site	328.7	2	7.072(**)	43.39	2	7.947(***)
	Residuals	5508.0	237		775.33	284	

Appendix 5. Growth multiple comparison test for interaction using Wald Chi-square statistic. *Pval* significance code ("***" < 0.0001 < "**" < 0.001 < "*" < 0.05 < "." < 0.1 < " " < 1)

Apêndice 5. Teste de comparação múltipla de crescimento para interação usando a estatística de Qui-quadrado de Wald. Código de significância *Pval* ("***" < 0,0001 < "**" < 0,001 < "*" < 0,05 < "." < 0,1 < " " < 1)

Species	Fixed	pairwise	Height			Diameter		
			Value	Df	Chisq(Pval)	Value	Df	Chisq(Pval)
BEPE	KRAL	Lisbon-Sintra	-23.392	1	18.760	-6.532	1	23.209(***)
	NORD	Lisbon-Sintra	-10.625	1	3.871 (*)	-5.211	1	12.114(***)
	UNIT	Lisbon-Sintra	-12.417	1	5.286 (*)	-6.484	1	23.869(***)
	Residuals			96			111	
	Lisbon	KRAL-NORD	-13.607	1	6.348 (.)	-0.901	1	0.255
	Lisbon	KRAL-UNIT	-4.441	1	0.676	-0.560	1	0.116
	Lisbon	NORD-UNIT	9.167	1	2.881	0.341	1	0.037
	Sintra	KRAL-NORD	-0.841	1	0.024	0.420	1	0.197
	Sintra	KRAL-UNIT	6.535	1	1.464	-0.512	1	0.292
	Sintra	NORD-UNIT	7.375	1	1.865	-0.932	1	0.969
QURO	Residuals			96			111	
	FRAN	Lisbon-Sintra	-6.989	1	3.261	-2.614	1	6.0046(*)
	PAGO	Lisbon-Sintra	-4.375	1	1.278	-2.017	1	3.775
	POSA	Lisbon-Sintra	1.400	1	0.131	-3.905	1	13.397 (**)
	UNIT	Lisbon-Sintra	3.3088	1	0.731	-0.167	1	0.024
	Residuals			111			143	
	Lisbon	FRAN-PAGO	-1.347	1	0.121	-0.011	1	0.0001
	Lisbon	FRAN-POSA	-2.122	1	0.301	2.401	1	3.798
	Lisbon	FRAN-UNIT	-2.972	1	0.590	-0.383	1	0.097
	Lisbon	PAGO-POSA	-0.775	1	0.040	2.412	1	3.992
	Lisbon	PAGO-UNIT	-1.625	1	0.176	-0.372	1	0.095
	Lisbon	POSA-UNIT	-0.850	1	0.048	-2.784	1	5.107
	Sintra	FRAN-PAGO	1.267	1	0.107	0.585	1	0.452
	Sintra	FRAN-POSA	6.267	1	2.622	1.110	1	1.624
	Sintra	FRAN-UNIT	7.326	1	0.445	2.064	1	5.616
	Sintra	PAGO-POSA	5.000	1	1.669	0.525	1	0.363
	Sintra	PAGO-UNIT	6.059	1	2.451	1.479	1	2.883
	Sintra	POSA-UNIT	1.059	1	0.075	0.954	1	1.200
	Residuals			111			143	
QURU	FEST	Lisbon-Sintra	-3.333	1	0.316	-4.958	1	7.318 (**)
	VANA	Lisbon-Sintra	-9.818	1	2.740	-1.123	1	0.451
	Residuals			18			18	
	Lisbon	FEST-VANA	-1.500	1	0.064	0.584	1	0.068
	Sintra	FEST-VANA	-7.985	1	1.812	4.419	1	17.442(***)
All	Residuals			18			18	
	BEPE	Lisbon-Sintra	-15.478	1	30.924 (***)	-6.107	1	57.429 (***)
	QURO	Lisbon-Sintra	-1.664	1	0.476	-2.165	1	13.932(***)
	QURU	Lisbon-Sintra	-6.576	1	3.721	-3.073	1	7.623 (**)
	Residuals			237			237	
	Lisbon	BEPE-QURO	10.771	1	17.114 (***)	-0.830	1	0.956
	Lisbon	BEPE-QURU	13.409	1	18.567 (***)	1.1785	1	0.920
	Lisbon	QURO-QURU	2.638	1	0.799	2.008	1	3.269
	Sintra	BEPE-QURO	24.585	1	89.165 (***)	3.112	1	36.491(***)
	Sintra	BEPE-QURU	22.311	1	51.404 (***)	4.213	1	46.811(***)
	Sintra	QURO-QURU	-2.274	1	0.593	1.101	1	3.551
	Residuals			237			237	

Appendix 6. Temperature that causes death to 50% of plants TL50 ranking (°C) per provenance

Apêndice 6. Temperatura que provoca a morte de 50% das plantas TL50 ranking (° C) por proveniência

Provenance	TL50 (°C)
QURO-UNIT	-7,26
BEPE-NORD	-7,18
BEPE-KRAL	-7,15
QURO-POSA	-7,15
QURO-PAGO	-7,12
BEPE-UNIT	-6,82