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Plant height determines phenological variation in *Quercus suber* L.

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Abstract. Phenological traits of vegetative apical buds were monitored in a *Quercus suber* L. (cork oak) *montado* near Lisbon during 2015. Natural regeneration plants, growing at a fenced plot, and mature trees (MT) from the surrounding *montado* were selected and divided in six groups (n=8) according to height (H): Sd1 (small seedlings, H<0.1 m); Sd2 (seedlings, 0.1<H<0.5 m); Sp (saplings, 1<H<2 m); Jv1 (small juveniles, 3<H<6 m); Jv2 (juveniles, H>6 m); MT (mature trees). Observations included budburst date, apical shoot elongation and leaf production.

Vegetative phenology patterns of *Q. suber* changed according to height/age group. Average budburst dates occurred between early-April (day 99, Sd1) and mid-May (after day 120, mature and juvenile trees), at day lengths between 12.8 (Sd1) and 13.7 hours (Jv2). Height was positively related with average budburst dates, degree day sums and daylength at budburst. Shoot elongation followed different patterns according to size/age group. In seedlings, cumulative growth was smaller and restricted to the weeks immediately after budburst, whilst taller/older trees phenological patterns were more variable, with vegetative growth often maintained until mid-summer. The differences in budburst timing and vegetative growth patterns may be reflecting the different strategies to cope with resource limitation and maximize the length of the growing season among groups.

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Key words: Cork oak; Budburst date; Vegetative growth; Day length; Ontogeny.

Tamanho determina a variabilidade fenológica em plantas de *Quercus suber* L.

Sumário. Acompanhou-se o desenvolvimento fenológico de gomos vegetativos apicais num montado de *Quercus suber* L. (sobreiro), próximo de Lisboa, em 2015. Plantas da regeneração natural, de uma parcela vedada, e árvores adultas (MT) do povoamento circundante foram selecionadas e divididas em seis grupos (n=8), em função da altura (H): Sd1 (H<0,1 m); Sd2 (0,1<H<0,5 m); Sp (1<H<2 m); Jv1 (3<H<6 m); Jv2 (H>6 m); MT (árvores adultas). As observações incluíram a data de abrolhamento e o crescimento vegetativo (alongamento dos raminhos apicais e produção de folhas).

Os padrões fenológicos dos gomos vegetativos variaram de acordo com o grupo de altura/idade. O abrolhamento decorreu entre o início de abril (dia 99, Sd1) e meados de maio (> dia 120, árvores adultas e juvenis), com duração média do dia entre 12,78 (Sd1) e 13,71 horas (Jv2). A altura das árvores relacionou-se positivamente com a data média de abrolhamento, os graus-dias acumulados e a duração do dia à data do abrolhamento. O padrão de crescimento vegetativo diferiu em função da altura/idade das plantas. Nas plantas com H<0,5 m, o crescimento acumulado foi menor e restringido às semanas imediatamente após o abrolhamento. Nas árvores maiores, os padrões fenológicos foram mais variados, mantendo-se frequentemente o crescimento até meados do verão. Nestes casos, as diferenças na data do abrolhamento e nos padrões de crescimento vegetativo podem estar a refletir diferentes estratégias para ultrapassar a limitação de recursos e maximizar a duração da época de crescimento.

Palavras-chave: Sobreiro; Data do abrolhamento; Crescimento vegetativo; Duração do dia; Ontogenia.

La taille détermine la variabilité phénologique des plantes de *Quercus suber* L.

Résumé: Le développement phénologique des bourgeons végétatifs apicaux a été suivi dans un *montado* de *Quercus suber* L. près de Lisbonne, en 2015. Des plantes de régénération naturelle, provenant d'une parcelle clôturée, et des arbres adultes (MT) provenant du peuplement environnant ont été sélectionnés et divisés en six groupes (n=8), en fonction de la hauteur (H): Sd1 (H<0,1 m); Sd2 (0,1<H<0,5 m); Sp (1<H<2 m); Jv1 (3<H<6 m); Jv2 (H>6 m); MT (arbres matures). Les observations ont porté sur la date de bourgeonnement et la croissance végétative (élongation des branches apicales et production de feuilles).

Les schémas phénologiques végétatifs de *Q. suber* changent en fonction de la hauteur et du groupe d'âge. Les dates moyennes de bourgeonnement ont eu lieu entre début avril (jour 99, Sd1) et mi-mai (> jour 120, arbres matures et juvéniles), à des durées de jour comprises entre 12,8 (Sd1) et 13,7 heures (Jv2). La hauteur était positivement liée aux dates moyennes de bourgeonnement, aux sommes de degrés-jours et à la longueur du jour au bourgeonnement. Le modèle de croissance végétative différait selon l'âge et la hauteur des plantes. Dans les plants avec H<0,5 m, la croissance accumulée était plus faible et limitée

à des semaines immédiatement après le bourgeonnement. Inversement, chez les arbres plus grands, le modèle de croissance végétative est plus variable y maintenue jusqu'au milieu de l'été. Entre les groupes, les différences dans la date de bourgeonnement et les schémas de croissance végétative peuvent refléter des stratégies différentes pour surmonter la limitation des ressources et maximiser la durée de la saison de croissance.
Mots-clés: Chêne-liège ; Date de bourgeonnement ; Croissance végétative ; Durée du jour ; Ontogénie.

Introduction

Quercus suber L. (cork oak) is a long-lived tree species, dominating vast sparse woodlands along the Western Mediterranean Basin, where it occurs across a wide range of climatic conditions (NATIVIDADE, 1950; ERIKSSON *et al.*, 2017). However, the long-term sustainability of cork oak systems is being threatened by biotic and abiotic stresses (ACÁCIO *et al.*, 2009; CARNICER *et al.*, 2011; CAMILO-ALVES *et al.*, 2013; SERRANO *et al.*, 2021), namely, the ongoing changes in precipitation and temperature (IPCC, 2022). Climate changes are inducing shifts in phenological patterns at global level (KÖRNER and BASLER, 2010; PIAO *et al.*, 2019; MENZEL *et al.*, 2020), with a consistent anticipation of leaf unfolding, as a consequence of rising spring temperatures, being observed in the last decades (PEÑUELAS and FILELLA, 2001; PEÑUELAS *et al.*, 2009). Changes in phenological timings can affect species and ecosystem functioning but our understanding of the intraspecific variation of these changes remains limited.

Q. suber is a late blooming species (ERIKSSON *et al.*, 2017), with budburst, leafing and shoot growth usually occurring during mid-spring, from April onwards (NATIVIDADE *et al.*, 1950; PEREIRA *et al.*, 1987; OLIVEIRA *et al.*, 1994; PINTO *et al.*, 2011; LOBO DO VALE *et al.*, 2019), later than other Mediterranean oaks (GARCÍA-MOZO *et al.*, 2002; JATO *et al.*, 2002; VARELA *et al.*, 2008; MORIN *et al.*, 2010; MANNAI *et al.*, 2017). Occasionally, in years with mild conditions, a less intense growth flush is observed in autumn (NATIVIDADE, 1950; PEREIRA *et al.*, 1987; OLIVEIRA *et al.*, 1994; FIALHO *et al.*, 2001; PINTO *et al.*, 2011; ERIKSSON *et al.*, 2017). The influence of environmental conditions on bud development and vegetative growth of *Q. suber* trees is well documented (OLIVEIRA *et al.*, 1994; FIALHO *et al.*, 2001; PINTO *et al.*, 2011; COSTA E SILVA *et al.*, 2015; LOBO DO VALE *et al.*, 2019). Whilst air temperature and rainfall are generally assumed to be the main triggers of *Q. suber* budburst timing and growth (PINTO *et al.*, 2011; COSTA E SILVA *et al.*, 2015; LOBO DO VALE *et al.*, 2019), with budburst being anticipated by warmer temperatures and delayed by reduced rainfall, the degree of control exerted by factors like photoperiod remains uncertain (AGUADO *et al.*, 2017; FERNÁNDEZ *et al.*, 2008; MARCHIN *et al.*, 2015). Similarly, the degree of variation deriving from tree characteristics, such as height or age, are yet to be addressed.

Even though budburst timing of trees depends on species (AUGSPURGER, 2008), within the same species it is known to vary according to tree height because of ontogenetic effects (AUGSPURGER and BARTLETT, 2003; VITASSE 2013). However, most studies addressing *Q. suber* vegetative phenology were based on

even tree age/size groups, including only saplings (SAMPAIO *et al*., 2016), juvenile (FIALHO *et al*., 2001; LOBO DO VALE *et al*., 2019) or mature trees (PINTO *et al*., 2011; COSTA E SILVA *et al*., 2015), or included trees of different ages growing at different sites (MOLINAS *et al*., 1992). Although growth traits of potted seedlings have been studied under different light, temperature, and water availability regimes (FERNÁNDEZ *et al*., 2008; AGUADO *et al*., 2017), the phenological patterns of natural regeneration seedlings remain mainly unknown. To our knowledge there are no previous studies addressing budburst and vegetative phenology of *Q. suber* across a height gradient, from seedling stage to adult trees (as a proxy of age), in plants growing in natural conditions at the same site.

This study aims to disclose the intra-specific variability of budburst and vegetative growth patterns of *Q. suber*, from the seedling stage to mature trees, growing under natural conditions at the same site, in Central Portugal, during a particularly dry year. Natural regeneration plants, selected from a fenced plot left largely unmanaged for approximately 15 years, and mature trees (MT) from the surrounding woodland were selected according to a height (H) gradient. Bud development was regularly monitored, and budburst dates registered. Subsequently, apical shoot elongation and leaf production were periodically assessed until the end of the growing period. We analysed the relationships between height/age and budburst date, day length, and duration of budburst and elongation periods aiming to understand the role of ontogeny in *Q. suber* vegetative phenology. We hypothesize that *Q. suber* has a significant intra-specific variation in phenological patterns and that plants of different height groups will respond differently to environmental conditions.

Materials and methods

Experimental site

The study was conducted in a large pure *Quercus suber* L. woodland in Central Portugal, ca. 40 km from Lisbon (38º 50′ N; 8º 49′ W), hereafter referred to as "Lezírias". The experimental site is a sparse *montado*, ca. 30 trees ha^{-1} and tree crown cover of 29%, with an understory composed almost exclusively of grassland species. Site topography is flat, and the soil is a well-drained deep Haplic Arenosol (IUSS WORKING GROUP, 2006) with a low water retention capacity. A shallow water table overlies a thick clay layer at 9 m depth.

Climate is of Mediterranean type with wet, mild winters and dry, hot summers. Long-term (1971-2000, for a near meteorological station, Coruche) mean annual rainfall is 622 mm $year^{-1}$ (mainly concentrated from October to April) and mean annual air temperature is 16.0 ^{o}C (ranging from 9.1 ^{o}C in January to 22.6 ^{o}C in July) (Instituto Português do Mar e da Atmosfera, Lisboa).

Plant material

Observations were done in six groups of plants of different sizes, including seedlings, saplings, juvenile and mature trees.

Seedlings, saplings, and juvenile trees were selected from natural regeneration plants growing in a fenced plot (ca. 42 x 31 m), protected from grazing, and left largely unmanaged since the year 2000. Mature trees were randomly selected from the surrounding woodland. The sampling groups were defined according to plant height (*H*, m) and plant availability: a group being formed when a minimum of eight trees of similar height, at least 2 m apart from the nearest neighbour, existed. The selected plants were divided in six different groups: Sd1 – small seedlings, $H < 0.1$ m; Sd2 - seedlings, *H*: 0.1 – 0.5 m; Sp - saplings, *H*: 1-2 m; Jv1 – small juvenile trees, *H*: 3-6 m; Jv2 - juvenile trees, $H > 6$ m; MT - mature trees. Saplings and juvenile trees were below the legal perimeter (70 cm measured 1.3 m above ground level) for cork harvesting, whilst mature trees were under "*amadia* cork" (the cork that grows after the two initial harvestings) exploitation. No flower production was observed in the natural regeneration trees growing in the fenced plot. General information regarding the morphometric traits of the sampling groups is given in Table 1.

Q. suber is a resprouting species and plants that have lost their aboveground mass may resprout. To assure that sampling included small seedlings, plants in the Sd1 group were selected only when a decaying acorn could still be seen. Hence, although the exact age of the studied trees was not accessed, and considering the possibility that, among the taller natural regeneration plants (saplings and juvenile trees), height groups are not coinciding with age groups, sampling included plants in different life stages (seedlings, saplings, young trees up to 15 years, and mature trees). Therefore, it is possible to assume that monitoring was done along an age gradient and that eventual differences in vegetative phenology are also reflecting ontogenic changes.

Meteorological measurements

Air temperature and rainfall were continuously monitored at the site throughout 2015, with an aspired psychrometer (air temperature, H301, Vector Instruments, Rhyl, UK) and a tipping-bucket rain gauge (rainfall, ARG100, Environmental Measurements, Gateshead, UK). Measurements were recorded as 10-min averages (temperature, ^{o}C) or totals (rainfall, mm) with a CR10X data-logger (Campbell Scientific, Shepshed, UK).

Table 1 - General morphometric traits for the studied height groups. Values are means ± *se* (*n* = 8)

	Sd1 Small seedlings $H < 0.1$m	***Sd2*** Seedlings $0.1 < H < 0.5$ m	***Sp*** Saplings $1 < H < 2$ m	***Jv1*** Small juveniles $3 < H < 6$ m	***Jv2*** Juveniles $H > 6$ m	***MT*** Mature trees
Tree height (*H*, m)	0.0942 (±0.008)	0.328 (±0.035)	1.820 (±0.094)	5.021 (±0.161)	7.678 (±0.364)	12.488 (±0.258)
Diameter at stem base (Dsb, m)	0.0026 (±0.00015)	0.0028 (±0.0009)	0.0584 (±0.0015)	0.173 (±0.008)	0.279 (±0.011)	0.571 (±0.035)
Diameter at 1.30 m (*DBH*, m)	-	-	-	0.106 (±0.006)	0.198 (±0.009)	0.476 (±0.035)

Phenological measurements

Two phenological phases were considered: budburst date and vegetative growth (apical shoot elongation and leaf production) (Figure 1). Observations started in January 2015, following the selection, and labelling of apical vegetative buds from previous-year sun exposed shoots. Six buds, from different accessible branches of the upper two-thirds of the crowns, were monitored in each plant of all groups, except for the smaller seedlings (Sd1), as only one or two buds were available per plant. Bud sampling was done in the South-facing side of the crowns in mature and juvenile trees and in the entire crown in seedlings and saplings, because of the smaller number of apical buds.

The date of budburst was defined as the first sampling day (registered as day of year, DOY) when new leaves or leaf tips emerging from the bud were visible

(Figure 1c). Budburst length was estimated as the difference (number of days) between the budburst dates (DOY) of the first and last buds of each group reaching that phase. Following budburst, shoot elongation was measured as the increase in length (cm). For each bud, the length of the shoot elongation season was estimated as the number of days (DOY) between the budburst date and the last day when increases in shoot length were registered.

From early March to mid-June, periods with more intense vegetative phenological activity (bud development and shoot elongation), measurements were done once/twice a week. During the rest of the year, observations were done every 1-4 weeks. Elongation was assessed using a tape (error range ± 1 mm). After leafing, the number of leaves per shoot was also monitored in every observation day. Access to crowns was granted by fixed scaffold towers and/or portable ladders.

For each group, budburst date and budburst length were estimated as the average of the budburst dates/lengths of all the sampled buds from the same group. Annual shoot elongation, leaf production and length of the shoot elongation season were also averaged for each group from all the sampled twigs, considering averages per tree.

Figure 1 – Examples of phenological stages observed on *Q. suber* vegetative buds during 2015: dormant bud (a), swelling bud (b); single bud budburst: day of the year (DOY) with visible leaf tips emerging from the bud (c); Shoot elongation and leaf unfolding (d); shoot elongation and leaf expansion (e); shoot with two growth flushes, spring and summer, and the respective leaf cohorts (f)

Bud development and temperature

Temperature needs to complete bud development (degree-day sums, DDS, ºC d) were estimated considering 6.2 ºC as base temperature (T_b, ºC) and 1 January and 25 March as the assumed days to break bud dormancy and start thermal unit accumulation, following previous estimations obtained for mature *Q. suber* trees at a nearby plot (PINTO *et al*., 2011).

DDS needed to reach budburst were estimated from daily average temperatures (ºC), as the sum of the differences between the daily mean air temperature and the base temperature (HUNTER and LECHOWICZ, 1992). The assumed base temperature is within the range of T_b reported by several authors (JATO *et al*., 2002; SAMPAIO *et al*., 2016) for bud development of evergreen Mediterranean oaks. The starting dates for DDS accumulation were decided based on PINTO *et al*. (2011).

Day length estimation

Day length or photoperiod (day-light hours) for a specific day was estimated as recommended by ALLEN *et al*. (1998). The average day length of budburst for each group was estimated as the average for all the buds selected in the plants of that group.

Statistical analyses

Data was subjected to one-way Analyses of Variance (ANOVA), using SigmaPlot statistic software (SigmaPlot 13.0, Inpixon, Palo Alto, CA, USA), after testing Normality (Shapiro-Wilk) and Equal Variance (Brown-Forsythe). The ANOVA was performed to evaluate differences between groups of trees (mean budburst date, budburst duration, DDS, day length, apical shoot elongation and leaf production). When the assumptions for ANOVA were not met, Kruskal-Wallis non-parametric test and Dunn's method were used for multiple comparisons of means. The analyses were performed for a significance level of 5% (α=0.05).

Results and discussion

Air temperature and cumulative rainfall at the experimental site (Lezírias), measured during 2015 are presented in Figure 2. Annual mean air temperature was 16.4 ºC, slightly higher than the average for the 2007-2017 period (16.1 ºC), also measured at the site. Mean air temperature during the first four months of 2015 was slightly below the average (11.8 and 12.2 ºC, respectively). However, from March onwards, monthly averages of air temperature were consistently higher than the 11-year average, mainly reflecting maximum temperature.

Cumulative rainfall (R) during 2015 was 368.5 mm, ca. 62% of the site 11-year average (591.6 mm), the year being consistently dry regardless of seasons. In the first four months of the year, cumulative rainfall was 131.5 mm, approximately 50% less than the site 11-year average (260.7 mm).

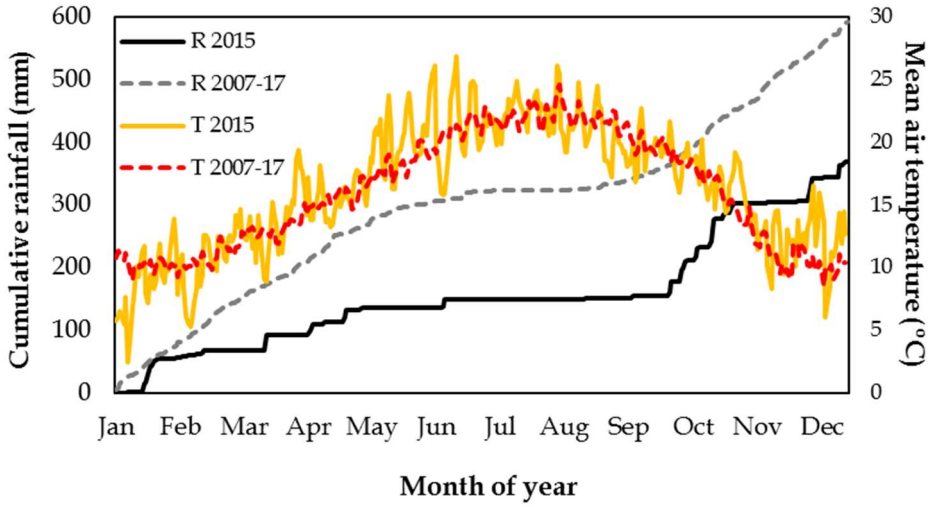

Figure 2 – Daily mean air temperature (T, yellow line) and cumulative rainfall (R, black line) at the study site during 2015. Dashed lines represent the averages of daily mean air temperature (red) and cumulative rainfall (grey) for the 2007-17 period

Budburst

Budburst occurred between early April (DOY 99, seedlings) and early May (DOY 122, juvenile and mature trees) (Table 2). This agrees with previous studies describing budburst, leafing and shoot growth of *Q. suber* to occur during mid-

spring, usually from April onwards (NATIVIDADE *et al*., 1950; PEREIRA *et al*., 1987; OLIVEIRA *et al*., 1994; PINTO *et al*., 2011; ERIKSSON *et al*., 2017; MANNAI *et al*., 2017). In our study, the onset and duration of budburst was not simultaneous between groups of trees (p<0.001) (Table 2, Figure 3a). Average budburst dates differed between both groups of seedlings, DOY 99 (Sd1) and 107 (Sd2) (p<0.05), being significantly earlier (p<0.05) than observed for taller trees (Table 2). Budburst of seedlings and saplings usually occurs earlier because they physiologically require less accumulation of degree-days of temperature for budburst (SEIWA, 1998, 1999; AUGSPURGER and BARTLETT, 2003). In Mediterranean-type climates earlier budburst in smaller plants, with less developed root systems, may be a developmental strategy to provide them opportunity to grow and gain carbon efficiently in the short period when environmental conditions (temperature and water availability) are more favourable and competition by resources is still limited. In taller trees, delayed budburst may be a safety mechanism to avoid the risks of late frosts in newly formed leaves, as reported for beech (GÖMÖRY and PAULI, 2011) or in reproductive organs (SEIWA, 1999) as the timing of flowering and vegetative bud emergence are almost identical. In *Q. suber*, it has also been shown to reduce leaf predation risks, as earlier budburst is associated with higher leaf pest damages (SAMPAIO *et al*., 2016). However, a delay on the onset of the vegetative season may have negative consequences on carbon assimilation and tree growth.

In our study, 2-3 weeks separate the average budburst dates of seedlings and juvenile/mature trees (p<0.001) (Table 2). This time span is shorter than that reported for Mediterranean oaks (*Q. ilex* and *Q. faginea*), where seedling budburst occurred one month before that of adult trees, likely reflecting differences in resource availability for leaf production and in carbon allocation patterns (MEDIAVILLA and ESCUDERO, 2009). Considering all plants and groups, single apical bud budburst dates spanned a period of six weeks, with the earliest observations registered at DOY 93 (Sd1 and Sd2 groups) and the latest at DOY 135 (Jv1 and MT groups) (Figure 3a). In smaller seedlings, Sd1, budburst was completed in a shorter time span (11 days, between days 93 and 104) than observed for each of the other groups (p<0.05) (occurring in 14 to 25 days intervals) (Figure 3a). Given the higher tree density of the natural regeneration plot, it is possible that varying microclimatic conditions, like temperature differences caused by shading from neighbour trees, may have affected bud temperature and, consequently, bud development, increasing variability in budburst (VITASSE *et al*., 2021).

Table 2 - Average budburst date (DOY), accumulated degree-day sums (DDS) and day length (h) at budburst day for the studied height groups. DDS estimation considered a base temperature of 6.2º C and different starting dates (1 Jan, DDS_{1Jan}, and 25 March, DDS_{25Mar}). Values are means ± se (n = 8).

	Sd1 Small seedlings $H < 0.1$m	***Sd2*** Seedlings $0.1 < H < 0.5$ m	***Sp*** Saplings $1 < H < 2$ m	***Jv1*** Small juveniles $3 < H < 6$ m	***Jv2*** Juveniles $H > 6$ m	***MT*** Mature trees
Mean budburst day (DOY)	99.1 [a] (±1.9)	107.4 [b] (±1.9)	116.5 [c] (±1.9)	122.6 [c] (±1.9)	122.8 [c] (±0.9)	121.4 [c] (±2.8)
DDS_{1Jan}	473.56 [a]	546.86 [a,b]	624.12 [b,c]	683.04 [c]	683.04 [c]	671.49 [c]
DDS_{25Mar}	140.20 [a]	213.50 [a,b]	290.76 [b,c]	349.68 [c]	349.68 [c]	338.13 [c]
Day length at budburst (h)	12.78 [a] (±0.08)	13.12 [b] (±0.08)	13.48 [c] (±0.07)	13.71 [c] (±0.07)	13.71 [c] (±0.03)	13.66 [c] (±0.10)

*Different letters indicate statistically significant differences (p<0.05).

The shorter time span observed for smaller seedlings budburst (p<0.05) may also be reflecting the strong dependence on environmental requirements for seedling budburst and the need to grow when conditions are favourable. Even though *Q. suber* leaf phenology has been shown to be under strong genetic control (SAMPAIO *et al*., 2016), trees may respond differently to environmental drivers between life stages (LEFÈVRE *et al*., 2014) as this trait is probably governed by different sets of genes/allels in younger and older ages. Hence, seedling budburst is likely to be under a stricter genetic control than older trees, with the shorter window for budburst reflecting a more "rigid" response to the environmental and physiological drivers involved. From an evolutionary perspective, it is possible to assume that selection should favour a more strict control of traits with important adaptation/survival effects (LEFÈVRE *et al*., 2014). A looser genetic control of bud development may be expected for older plants, with individual trees responding to the environmental triggers for budburst differently, some blooming consistently earlier or later between years (MALYSHEV *et al*., 2022). Additionally, taller/older plants are more likely to have increased nutrient and carbohydrate storage to support growth (KOZLOWSKI, 1992), which may decouple to some extent the dependence of budburst from meteorological conditions.

We found a positive relationship between tree height and average budburst dates, with smaller trees tending to have earlier average budburst dates than

taller ones (Figure 3b). This relationship is more relevant for trees under 2 m, suggesting the possible existence of a threshold beyond which budburst is less influenced by tree size. It can be hypothesized that the set of genes controlling this trait may be switched on/off for higher trees, with less resource limitation (*e.g.* roots reaching underground water sources) or with increased carbon storage. In temperate species, the influence of tree height on budburst date is not clear, with reports of positive (GÖMÖRY and PAULI, 2011) and negative relationships (MARCHAND *et al.*, 2020). However, MARCHAND *et al.*, 2020 observed a weak positive multi-species relationship between budburst and tree age.

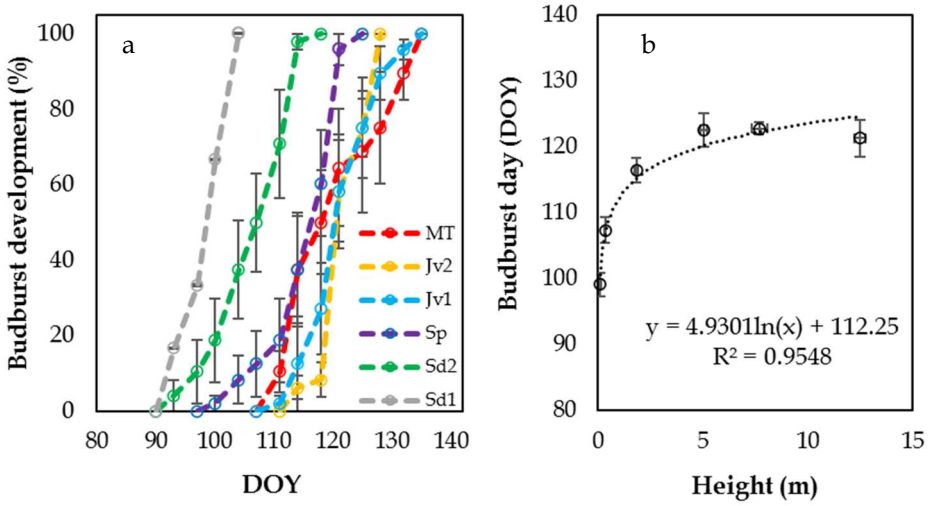

Figure 3 - Relative budburst development (%) (a), and relationship between average plant height and the average budburst day (b). Error bars are standard errors (n = 8)

Air temperature was found to be the main driver of budburst timing of Mediterranean oaks of the Iberian Peninsula (MORIN *et al.*, 2010; COSTA E SILVA *et al.*, 2015; SAMPAIO *et al.*, 2016; LOBO DO VALE *et al.*, 2019) and for mature *Q. suber* trees at Lezírias site (PINTO *et al.*, 2011). Average daily mean air temperature during the first 4 months of 2015 (11.8 ºC) was lower, by 0.14 to 0.92 ºC, than previously reported for the same site (PINTO *et al.*, 2011). This lower mean air temperature did not seem to affect the rate of bud development of taller/older trees, as the average budburst dates of mature and juvenile trees (Table 2) fell in the reported time interval (DOY 119 to 125) (PINTO *et al.*, 2011).

The rise in maximum air temperatures, observed from March onwards at Lezírias site, may have accelerated bud development in juvenile and mature trees and offset the effects of the colder previous months, as high temperatures in the preceding 1-3 months promote earlier leafing and flowering (WIELGOLASKI, 2001; MENZEL and SPARKS, 2006). Interestingly, at the same site, bud development of *Q. suber* adult trees has been found to be more effectively accelerated by high mean and maximum daily temperatures in periods close to budburst (late-March and April) than those of earlier periods (January and February) (PINTO *et al*., 2011). GARCÍA-MOZO *et al*. (2006) reported that air temperature and rainfall, one month prior to flowering, were the most influential drivers on oak phase timing. It is possible that the rate of bud development of seedlings and saplings was slowed by the lower mean January and February mean air temperature, as observed by SAMPAIO *et al*. (2016) for *Q. suber* saplings. The same authors also reported higher budburst date variability in the year with higher January-February temperature. The dependence of seedling budburst dates on winter temperature is likely a common feature for Mediterranean evergreen oaks, as leaf unfolding of *Q. ilex* seedlings has been found to be highly responsive to temperature, being significantly delayed in colder years (MORIN *et al*., 2010).

The influence of rainfall on *Q. suber* budburst timing has been less studied. Although PINTO *et al*. (2011) did not find any across-site relationship between rainfall and budburst date in *Q. suber*, COSTA E SILVA *et al*. (2015) and LOBO DO VALE *et al*. (2019) observed that budburst and leaf expansion were delayed by several weeks in dryer years. In the present study, average budburst dates of taller/older trees fell within the time interval reported by PINTO *et al*. (2011), for budburst of nearby mature trees studied in wet (DOY 119) and dry (DOY 124) years.

Budburst dates reflect the influence of photoperiod and accumulated thermal time (CANNELL and SMITH, 1983; JACKSON, 2009; MARCHIN *et al*., 2015), with higher trees tending to have to accumulate more thermal units than smaller ones. Thermal needs for budburst varied between height groups (p<0.001), regardless of the considered starting date (Table 2). Considering 1 Jan as starting date (DDS_{1Jan}), budburst of mature and juvenile trees occurred following the accumulation of 41 and 22% more thermal units than Sd1 and Sd2 plants, respectively. These results are in accordance with previous studies highlighting that seedlings and saplings are physiologically known to require less accumulation of degree-days of temperature for budburst (SEIWA, 1998, 1999; AUGSPURGER and BARTLETT, 2003). Between seedlings and older trees, the

proportional differences in thermal unit requirements for taller trees increased when the considered starting date approached the average budburst dates (DDS_{25Mar}) (over 60 and 70% more) (Table 2), reinforcing the importance of higher temperatures closer to budburst for *Q. suber* vegetative bud development (PINTO *et al*., 2011; GARCÍA-MOZO *et al*., 2006).

Photoperiod may also play an additional role in the triggering mechanisms of budburst, by constraining the influence of temperature to a safer period (MENZEL and SPARKS, 2006). In *Q. ilex* and *Q. faginea* seedlings, photoperiod has been shown to have a stabilizing effect on bud development by delaying the advancing effect of higher temperatures under shorter days (SANZ-PÉREZ *et al*., 2009). For *Q. suber* the dependency of budburst on photoperiod has been less studied. In our study, the average day length at budburst date varied between the height groups (p<0.001) (Table 2). Daylength at budburst differed between both groups of seedlings, 12.78 h (Sd1) and 13.12 (Sd2) (p<0.05), being significantly shorter (p<0.05) than observed for taller trees. In Jv1, Jv2 and MT groups, the average day length at budburst was ca. 13.7 h (Table 2). Smaller seedlings (Sd1) were the only group for which budburst occurred under less than 13 hours of day light, with the earliest buds breaking at day lengths of 12.5h. AGUADO *et al*. (2017) observed that budburst of 18-month-old potted *Q. suber* seedlings started at daylight durations over 12.2 h. In a previous study at Lezírias, over a four-year period, budburst of mature trees occurred at an average day length of 13.8 h (PINTO *et al*., 2011).

Vegetative growth

Cork oak has been reported to have an intense, but relatively short growth period, mainly concentrated in spring, sometimes with a less intense growth flush in autumn (NATIVIDADE, 1950; PEREIRA *et al*., 1987; OLIVEIRA *et al*., 1994; ERIKSSON *et al*., 2017), the seasons when water availability and air temperature are adequate to support them. In our study, all groups of plants had intense vegetative growth activity in the weeks following budburst (from mid-April onwards) (Figures 4 and 5), with some trees ceasing growth in mid-summer (Figures 4 and 5). A clear variability in current-year apical shoot elongation patterns was observed, both within and between groups of trees (Table 3, Figures 4 and 5). On average, cumulative apical shoot elongation varied between height groups (p<0.001), varying from 0.014 (Sd1) to 0.116 m (Sp). Similarly, we observed significant variation between height groups for the duration of vegetative growth (p<0.001), with *Q. suber* plants ceasing elongation between 12.8 (Sd1) and 60.6

(MT) days after budburst (Table 3, Figure 4). Saplings and smaller juvenile trees maintained shoot elongation for approximately six weeks, less than the 10 weeks reported by PEREIRA *et al*. (1987) for the duration of the spring flush of young *Q. suber* trees. In our study no autumn flush was observed, contrary to previous studies reporting a small period of less intense growth after summer in some years (PEREIRA *et al*., 1987; OLIVEIRA *et al*., 1994; PINTO *et al*., 2011). It has been suggested that the length of growing season might be proportional to the total annual carbon budget allocated for vegetative growth (CASTRO-DÍEZ *et al*., 2003). Although large plants allocate proportionally less carbon for growth, in favour of other processes (such as respiration) (KOZLOWSKI, 1992), it can be expected that their absolute carbon budgets for vegetative growth far exceed those of smaller individuals, resulting in prolonged and higher absolute growth, as observed in our study. However, average single shoot elongation represented less than 1% of total tree H for mature trees and taller juveniles (Table 3). In smaller plants, shoot elongation represented a higher percentage of total H, reflecting the smaller competition for carbon allocated for growth.

Prolonged sub-continuous floral and vegetative growth patterns (that is, alternating growth and rest periods in the same annual cycle) have been observed for some *Q. suber* trees (BARROS GOMES, 1876 cited by NATIVIDADE, 1950; ERIKSSON *et al*., 2017). Similarly, two types of shoot elongation patterns have been described for nearby mature *Q. suber* trees (PINTO *et al*., 2011), through proleptic (delayed) branching and through immediate branching (BARTHÉLÉMY and CARAGLIO, 2007). In delayed branching, current-year shoot growth was restricted to the weeks following budburst in buds developed in the previous year. In the second case, current-year shoot growth occurred in two consecutive and distinctive shoot growth phases, with a first spring growth flush, occurring immediately after budburst, and a second growth period, occurring in late-spring/early summer after a small rest phase. In this case, newly produced buds (just apical or apical and axillary) originated a new set of stems and a new leaf cohort (through immediate branching) and continued elongation until mid-summer (Figures 4 and 5).

Table 3 - Average values of apical shoot elongation (m), number of days after budburst to complete shoot elongation, percentage of growth relative to total tree height, percentage of monitored buds with two separate flushes (elongation/leaf production phases) and number of produced leaves per bud (first and second leaf cohorts) for each height group. Values are means ± se (n = 8).

	Sd1 Small seedlings H < 0.1m	***Sd2*** Seedlings 0.1< H < 0.5 m	***Sp*** Saplings 1< H < 2 m	***Jv1*** Small juveniles 3< H < 6 m	***Jv2*** Juveniles H > 6 m	***MT*** Mature trees
Apical shoot elongation (m)	0.014 [a] (±0.003)	0.045 [a,b] (±0.009)	0.116 [c] (±0.007)	0.093 [b,c] (±0.013)	0.059 [a,b,c] (±0.007)	0.109 [b,c] (±0.019)
Growth relative to total tree H (%)	16.3 (±2.65)	13.5 (±2.71)	6.4 (±0.46)	1.9 (±0.29)	0.8 (±0.10)	0.9 (±0.14)
Days to complete shoot elongation	12.8 (±1.2)	27.0 (±3.2)	42.4 (±9.7)	41.8 (±11.2)	22.6 (±3.0)	60.6 (±17.3)
% of buds with 2 flushes	0.00	0.00	31.25	12.50	4.17	33.33
Number of leaves 1st cohort	5.00 (±0.78)	8.33 (±0.87)	14.25 (±1.12)	10.54 (±0.99)	9.50 (±0.22)	10.13 (±0.51)
Number of leaves 1st + 2nd cohort	5.00 (±0.78)	8.33 (±0.87)	21.67 (±3.60)	14.75 (±2.11)	9.69 (±0.27)	17.79 (±3.85)

*Different letters indicate statistically significant differences in cumulative apical shoot elongation (p<0.05).

In our study, seedlings grew only through delayed branching whilst buds from some saplings and taller trees (except Jv2) developed through delayed and immediate branching (Figures 4 and 5). MT and Sp individuals showed the most variation in shoot elongation patterns, with over 30% of the monitored buds exhibiting two distinctive growth periods, some maintaining shoot elongation and leaf production until mid-summer (over 100 days after budburst) (Table 3, Figures 4 and 5). Although prolonged shoot elongation periods have been reported for some mature (OLIVEIRA *et al*., 1994; PINTO *et al*., 2011) and very young (FIALHO *et al*., 2001) *Q. suber* trees, it does not seem to affect all trees or be a general feature across stands (CARITAT *et al*., 1992), likely being restricted to mild/wet years (LOBO DO VALE *et al*., 2019) or sites with unrestricted access to deeper water sources. In a previous study at Lezírias, DAVID *et al*. (2013) observed that deep root access to groundwater allowed nearby mature *Q. suber* trees to maintain a favourable and spatially homogeneous water status throughout the summer months, regardless of rainfall distribution patterns. In

sites with shallow water tables and no limitations for the development of deep root systems, the high predawn leaf water potentials allow the maintenance of high cell turgor pressure, fulfilling the baseline requirements for growth (HSIAO, 1973). Hence, under these conditions, water supply should not constrain shoot elongation in trees (KUMMEROW, 1981). In sites where roots have limited or no access to deep water sources, *Q. suber* shoot elongation is more restricted and dependent on water availability and temperature prior to budburst (MOLINAS *et al.*, 1992; PINTO *et al.*, 2011). However, it is possible that the varying intra-group shoot elongation patterns observed in our study, are reflecting differences on photosynthetic capacities (USCOLA *et al.*, 2015) or carbohydrate reserves (GILSON *et al.*, 2014) to support the new growth, or result from the genetic variability among trees (SAMPAIO *et al.*, 2021). The existence of significant intra-population genetic variation and genetic control for traits such as growth has been shown for cork oak (RAMÍREZ-VALIENTE *et al.*, 2015; SAMPAIO *et al.*, 2021).

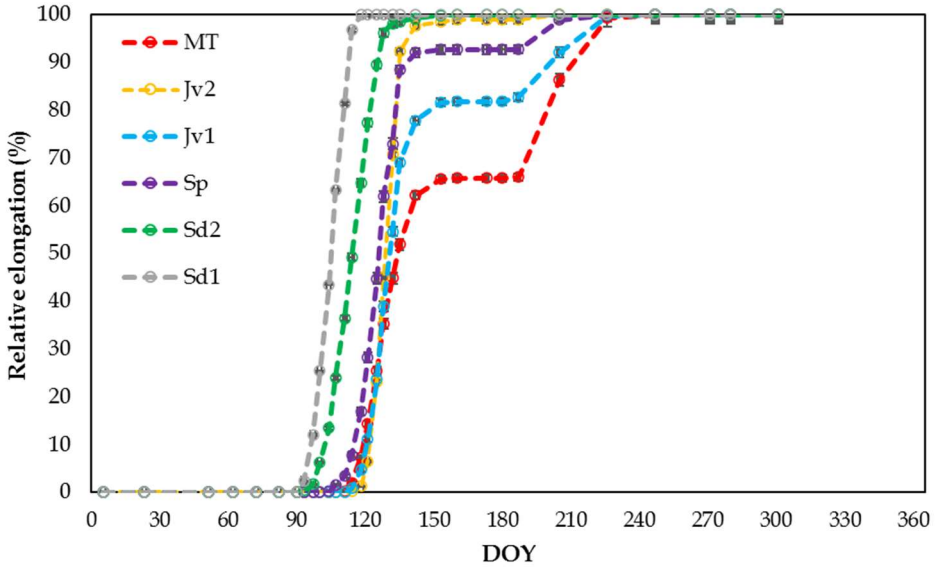

Figure 4 - Average apical shoot elongation (%, relative to maximum) after budburst day for each height group (dashed lines with symbols). Error bars (n = 8) are contained within the symbols

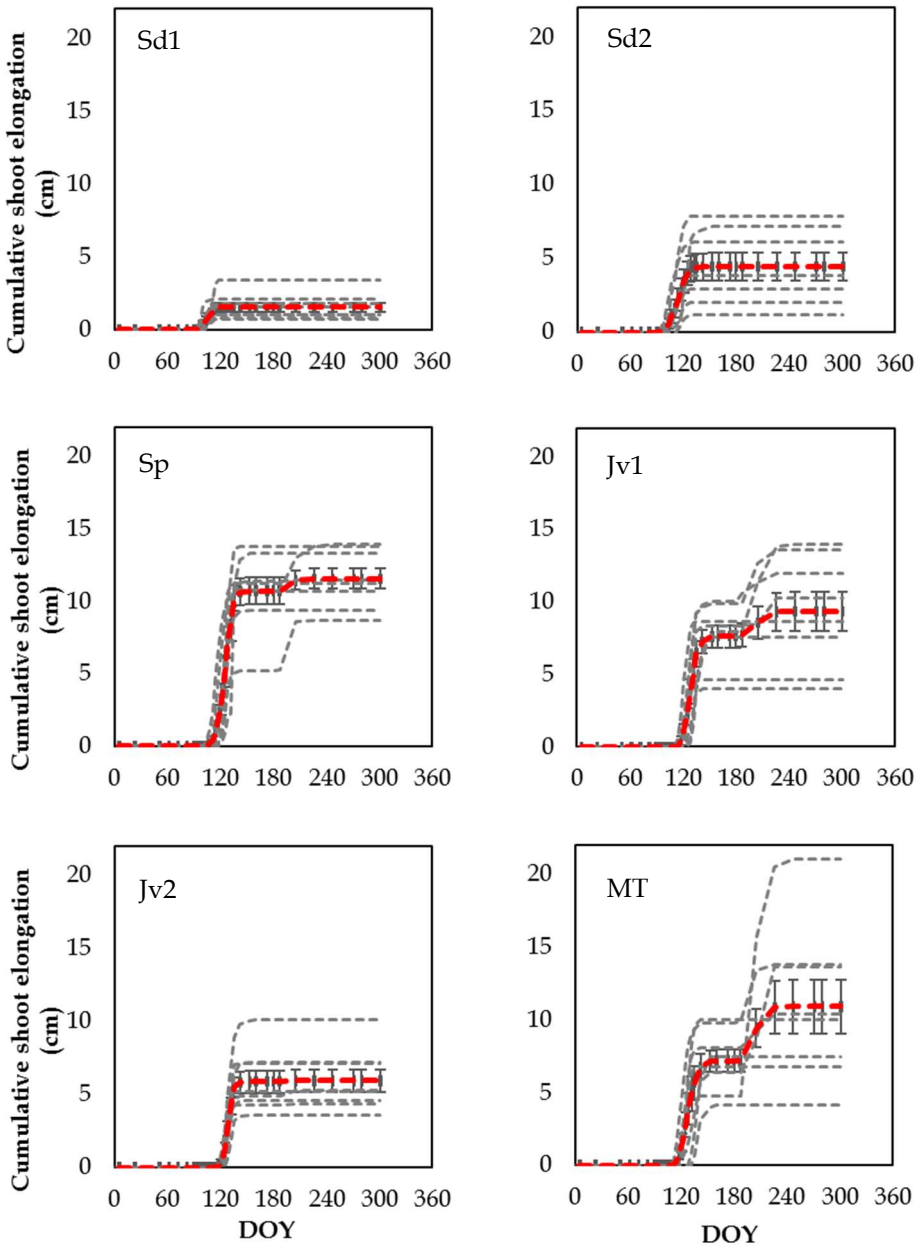

Figure 5 - Cumulative mean apical shoot elongation (cm) for the studied height groups during 2015. Dashed red lines represent the average shoot elongation for each group (n=8). Error bars represent standard errors. Dashed grey lines represent the mean value for each individual tree (6 observations per tree, except for Sd1)

In juvenile trees most buds developed through delayed branching, along 3-6 weeks, with only 12.40 (Jv1) and 4.17% (Jv2) of the monitored buds growing in two phases (Table 3). Shoot elongation in Jv2 was restricted to ca. three weeks, with shorter (0,059 m) new shoots than mature individuals and other juveniles (Table 3, Figure 5). Short apical shoot elongation (under 5 cm), completed in ca. four weeks was also reported for juvenile *Q. suber* trees (CARITAT *et al*., 1988; MOLINAS and CARITAT, 1989). However, contrary to what was observed in this study, juvenile trees growing in Catalonia were reported to have more vigorous shoot elongation than mature trees, although results might be reflecting different water availability among sites (MOLINAS *et al*., 1992). Considering the whole tree life span, radial growth rates of *Q. suber* are at their highest when the trees are around 15-25 years (NATIVIDADE, 1939). Similar findings were reported by CARITAT *et al*. (1992) for *Q. suber* in NE Spain, where juvenile trees had higher radial growth than mature trees. As our experimental plot was left unmanaged since the year 2000, and the Jv2 group included the tallest natural regeneration trees, it is possible to assume that trees in this group were approaching the age of maximum radial growth rates. Hence, the smaller cumulative vegetative growth observed in Jv2 trees may be reflecting different priorities for carbon allocation among groups of trees, with the channelling of resources for radial growth instead of vegetative growth.

Seedlings had the smallest cumulative shoot elongation, 0.014 (Sd1) and 0.045 m (Sd2), occurring in a single short flush completed in the weeks immediately after budburst (Figures 4 and 5, Table 3). However, it represented over 16% and 13%, Sd1 and Sd2, respectively, of plant height at the beginning of the experiment (Table 3). Nevertheless, it is possible that the apparently reduced vegetative growth of *Q. suber* seedlings is a likely consequence of prioritizing resources for root growth during the first stages of plant life (NATIVIDADE, 1941; MAROCO *et al*., 2002). Despite this investment, seedling root systems are restricted to upper soil layers, rendering growth more dependent on rainfall patterns and soil water availability. *Q. suber* seedlings have been found to support spring growth on stored and current C and N (CERASOLI *et al*., 2004). However, during 2015, the favourable time span for photosynthesis and carbohydrate production was limited by adverse environmental conditions (temperature and water availability) before and after budburst. Vegetative growth occurring in several separate flushes has been described for seedlings and saplings of Mediterranean oaks (MEDIAVILLA and ESCUDERO, 2009), a pattern ascribed to low carbohydrate reserves to supply leaf production at the beginning of the growing season. This was also observed in temperate oak seedlings growing in

experimental conditions, where the carbohydrates produced by the new spring leaves were directly translocated to support the second growth flush (DICKSON, 1989). Differences in stored and newly acquired nitrogen and carbohydrates have been shown to be positively related with vegetative growth of *Q. ilex* seedlings (USCOLA *et al*., 2015).

As observed in our study (Table 3, Figure 5), *Q. suber* current-year shoot elongation patterns are known to vary widely with site conditions or between years (CARITAT *et al*., 1992; MOLINAS *et al*., 1992; FIALHO *et al*., 2001; PINTO *et al*., 2011; LOBO DO VALE *et al*., 2019). The influence of plant age is not so clear, with studies reporting more pronounced shoot elongation in young (MOLINAS *et al*., 1992) or mature (OLIVEIRA *et al*., 1994) trees. However, in the study by MOLINAS *et al*. (1992) the different groups of trees were growing in different sites, with younger trees likely benefiting from more favourable rainfall patterns.

Saplings had the higher average cumulative shoot elongation, with 0.116 m (Table 3). This greater investment in shoot elongation and leaf production may be a possible strategy to maximize leaf area and photosynthetic capacity. Whilst *Q. suber* seedling survival is directly benefited by tree canopy cover (CALDEIRA *et al*., 2014), at sapling stage *Q. suber* plants might be prioritising height growth to overcome competition for light with understory shrubs.

Shoot elongation and leaf production were found to be highly correlated to climatic conditions, namely rainfall and air temperature (PINTO *et al*., 2011; LIMOUSIN *et al*., 2012). However, from May to August, when shoot elongation and leaf production were more intense, only 22.5 mm of rainfall were recorded, less than 30% of the 11-year average (67.4 mm) (Figure 2). In a 4-year study in nearby mature *Q. suber* trees, PINTO *et al*. (2011) reported elongation to vary between 0.061 and 0.222 m, for the driest and wettest years, respectively. In the present study, average shoot elongation for the taller plant groups remained closer to the lower range of the interval (Table 3, Figure 5) and below the values reported by LOBO DO VALE *et al*. (2019) for juvenile trees growing in southern Portugal during a mild and a dry year. This suggests that the dry winter and spring registered at Lezírias during 2015 (Figure 2) might also have contributed to restrict vegetative growth.

The average of the maximum number of new leaves produced by a single bud varied between 5.0 (Sd1) and 21.7 (Sp) (Table 3), with juveniles producing more leaves in Lezírias than in other sites (OLIVEIRA *et al*., 1994; LOBO DO VALE *et al*., 2019). Leaf production of mature *Q. suber* trees has been found to follow closely shoot elongation patterns and, consequently, depend on cumulative rainfall and air temperature conditions prior to budburst (PINTO *et al*., 2011).

MEDIAVILLA and ESCUDERO (2009) found no relationship between the number of flushes and the total number of leaves produced by seedlings and saplings of *Q. ilex* and *Q. faginea.* In our study, maximum individual bud leaf production was highly variable, differences were only significant between Sd1 and Sp (p<0.001), mainly reflecting each bud's number of flushes. Some buds, with growth restricted to the weeks immediately after budburst, produced only a small number of new leaves in spring. On the opposite, some buds with two growing phases produced several dozens of new leaves, increasing leaf area and photosynthetic capacity. Although, no significant differences were observed between cohorts, from the first to the second leaf cohort, the average number of produced leaves increased by ca. 40, 53 and 76%, in Jv1, Sp and MT groups, respectively (Table 3), with some individual buds exhibiting 5 to 10-fold increases in the number of leaves. The first leaf flush of Mediterranean oak (*Q. ilex* and *Q. faginea*) seedlings and saplings has been found to be the most abundant, usually producing approximately 50% of the total number of leaves (MEDIAVILLA and ESCUDERO, 2009).

Although we did not measure leaf area, during leaf counting, it was often noticeable that leaves produced in the second growth period were smaller and not always fully expanded, especially in Sp and Jv1 groups. Young *Q. suber* trees were reported to produce more leaves and with more leaf area in the spring flush than in the second (autumn) flush (PEREIRA *et al*., 1987).

Given the reduced size of the experimental plot and the evenly distribution of natural regeneration plants within it, it is not likely that the observed intra- and inter-group variability in shoot elongation is arising from micro-site differences in climate conditions, soil physical and chemical properties or in access to deeper water sources. Additionally, individual bud sampling and assessment was homogeneous for all groups (except Sd1, because of plant size). Hence, it is possible that, in young trees, the differences in budburst timing and vegetative growth patterns are reflecting differences in the capacity to support growth under environmental constraints, changes in genetic control of growth traits and varying ontogenic priorities for nutrient and reserve allocation. Dynamic carbohydrate allocation for growth guarantees that, at each life stage, the most critical responses to cope with environmental constraints are met. In mature trees, however, the variation in phenological patterns is more likely to be reflecting genetic variability and each tree's capacity to maintain growth under the multiple environmental stresses occurring at the same time (DICKSON, 1989).

Conclusions

Our results show that vegetative phenology of *Q. suber* varies according to height. Under field conditions, height is positively related with average budburst dates, degree day sums and daylength at budburst. Budburst of smaller seedlings seems to be under a strict genetic control, being tightly dependent of meteorological conditions, maximizing photosynthesis and growth in the most favourable periods. Shoot elongation patterns were widely variable both within and between height groups. Although seedling budburst dates occur earlier, shoot elongation is restricted to a few weeks. Ongoing reductions of spring rainfall may narrow the favourable window for growth, compromising further *Q. suber* seedling establishment success.

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