

Inter-Specific Differences in Leaf Phenology, Growth of Seedlings of Cork OAK (*Quercus suber* L.), Zeen Oak (*Quercus canariensis* Willd.) and Their Hybrid Afares Oak (*Quercus afares* Pomel) in the Nursery

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Abstract—Leaf Life Span (LLS) is used to classify trees into two main groups: evergreen and deciduous species. It varies according to the forms of life between taxonomic groups. Co-occurrence of deciduous and evergreen oaks is common in some Mediterranean type climate areas. Nevertheless, in the Tunisian forests, there is not enough information about the functional inter-specific diversity among oak species, especially in the mixed stand marked by the simultaneous presence of *Q. suber* L., *Q. canariensis* Willd. and their hybrid (*Q. afares*), the latter being an endemic oak species threatened with extinction. This study has been conducted to estimate the LLS, the relative growth rate, and the count of different growth flushes of samplings in semi-controlled conditions. Our study took 17 months, with an observation's interval of 4 weeks. The aim is to characterize and compare the hybrid species to the parental ones. Differences were observed among species, both for phenology and growth. Indeed, *Q. suber* saplings reached higher total height and number of growth flushes than *Q. canariensis*, while *Q. afares* showed much less growth flushes than the parental species. The LLS of parental species has exceeded the duration of the experiment, but their hybrid lost all leaves on all cohorts. The short LLSs of hybrid species are in accordance with this phenology in the field, but for *Q. canariensis* there was a contrast with observations in the field where phenology is strictly annual. This study allowed us to differentiate the hybrid from both parental species.

Keywords—Leaf life span, growth, hybrid, evergreen, deciduous, seedlings, *Q. afares* Pomel, *Q. suber* L., *Q. canariensis* Willd.

I. INTRODUCTION

AMONG the traits that determine the adaptation of trees species to their environment, phenology which corresponds to the occurrence of cyclical biological events

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related to seasonal variations in climate plays an essential role [1]. Leaf longevity, an important plant trait linking leaf ecophysiology and phenology is a quantitative functional trait [2], which varies among species from a few weeks to several years.

LLS [3] is the time period during which an individual leaf (or a leaf analogue) is alive and physiologically active and able to assimilate carbon. It allows to separate trees species into two main groups: Evergreen species, which keep leaves for several years, and deciduous species which keep leaves only during the growing season and replace them with a new set during the next growing season.

LLS varies significantly according to the life forms between the taxonomic groups and along the successional gradient [4], [5]. Therefore, it is a trait that is largely genetically determined [6]. In general, early successional species have a shorter LLS [7], [5] than late successional species.

Several studies have evidenced a trade-off between leaf production and maintenance. The 'cost-benefit hypothesis' asserts that long-lived leaves are more costly for maintenance, defense and less productive than short-lived leaves [8], [9]. To explain this relationship, a simple model with photosynthesis rate, construction cost and maintenance costs as parameters was proposed [10], [11]. It bases on the idea that a tree replaces its leaves to maximize carbon gain.

Species that have leaves with a longer life span show a lower level of total nitrogen, chlorophyll and soluble proteins [12]-[14] in comparison to species that have leaves with a shorter LLS.

Deciduous species are more sensitive to cold and frost than evergreen species [15]-[17].

To survive, species with longer LLS must have sufficient structural rigidity to withstand desiccation in winter [18] and must also resist against herbivory, hence production of secondary compounds (lignin, tannin, etc.) as well as the production of supporting tissues is favored.

The emergence and development of leaves depends on endogenous and exogenous factors. The most important exogenous factors seem to be the winter and spring temperatures and photo period [19]. Mediterranean flora is characterized by a large variability in leaf phenology among life forms [20].

In order to understand physiological ecology relationships between the hybrid and their parent species, it is pertinent to

have knowledge of their leaf habitats at seedling age.

This study has been conducted to estimate and compare the count of different growth flushes, the inter-specific diversity of LLS, the relative growth rate and the count of different growth flushes in samplings of the two species (*Q. suber* and *Q. canariensis*) and their hybrid (*Q. afares*) under semi-controlled conditions.

II. EXPERIMENTAL DETAILS

A. Materials and Procedures

The experiment was conducted at the nursery of INRGREF at Ariana, Tunisia (36.510° N, 10.120° E, 15 m asl), characterized by a semi-arid bioclimate (warmer and dryer winter compared to the origin of the seeds). It started in the winter of 2009 using the acorns of the three-species collected from native populations of *Q. canariensis* Willd., *Q. suber* L. and *Q. afares* Pomel, in NW Tunisia, where these species coexists in the Ain Zena massif (36.43° N, 8.51° E). Seeds were germinated in plastic pots filled with a substrate composed of peat, organic layer from a cork oak forest soil and sand. Pots were kept under external conditions. At the age of one year, the seedlings were transplanted to 10 L plastic pots filled with a substrate composed of peat, organic layer from a cork oak forest soil and sand (1/2/2 v/v/v). The seedlings were watered regularly. For measurements, 30 plants were used per species.

B. Calculations of the Number of Growth Flushes in Samplings and the LLS

Leaves of three species of *Quercus* were repeatedly surveyed for 17 months. New leaves developed and old leaves senesced throughout the year, yielding a continual replacement of leaves and a summer peak in leaf number.

A cohort of leaves (growth flush) was identified on the main axis for all the individuals for each species at the beginning of the experiment (date t₁). The first cohort (oldest cohort) was marked, and the number of initial leaves was determined. Oak species exhibit a rhythmic shoot elongation with successive growth/rest phases of fixed period as described by [21], [22]. The different growth flushes observed were distinguished by making with colored wires and were counted per species. Therefore, on each plant and for each monthly survey first the number of cohorts was determined and then the cumulative number of leaves per seedling and per cohort was recorded.

At the end of the experiment (after 17 surveys), most of the individuals (especially in the case of *Q. suber* and *Q. canariensis*) still retained leaves from the marked cohorts. For these cases, we used the T₅₀, which is the duration between onset and disappearance of 50% of the leaves of the cohort and was determined from the evolution curve of the number of leaves (finals/initials: FI/IN) as a function of time.

C. Estimation of the Relative Growth Rates and the Number of Flushes in Samplings

The relative growth rates in height (RGR_H, cm cm⁻¹.year⁻¹) and in the basal diameter (RGR_{BD}, mm mm⁻¹.year⁻¹) of each

seedling per species were estimated as:

$$RGR_H = ((\ln H_{t_2} - \ln H_{t_1}) / (t_2 - t_1)) \times 12 \quad (1)$$

$$RGR_{BD} = ((\ln D_{t_2} - \ln D_{t_1}) / (t_2 - t_1)) \times 12 \quad (2)$$

where H_{t₁} and D_{t₁} and H_{t₂} and D_{t₂} represent the height or diameter at time t₁ and t₂, respectively, and t₂-t₁ is the time interval (days). We used ruler to measure the height and caliper to measure two perpendicular diameters of seedlings. From two diameters, the mean diameter was calculated.

D. Statistical Analyses

Statistical analyses were performed using the software R. The data of relative growth rates and the number of flushes were subjected to an analysis of variance and the means compared for significant differences by Tukey' test (p<0.05).

III. RESULTS AND DISCUSSION

A. Differences in the Number of Growth Flushes per Year

A large interspecific variability was observed in the number of growth flushes among species (F = 212.5, P < 0.0001). *Q. suber* seedlings produced a large number of flushes (7/year), and reached a higher mean shoot length than *Q. canariensis*, which showed significantly less flushes (5/year), while *Q. afares* produced the lowest number of flushes (2 /year; Fig. 1).

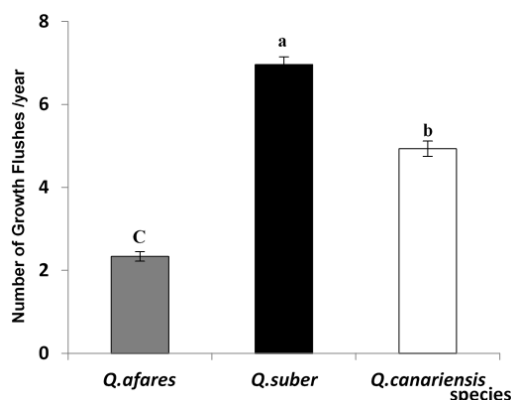


Fig. 1 Mean (± SE) number of flushes of growth on seedlings of the three species (bars are standard errors)

B. Interspecific Differences in LLS

Senescence of leaves occurred throughout the year but was faster and reached the maximum in winter. Accordingly, *Q. afares* (deciduous species) lost leaves on most cohorts (Fig. 2 (c)). While for *Q. suber* and *Q. canariensis*, a certain stability of the number of leaves was noted and only a small fraction of the leaves fell at the end of the experiment on the first cohorts as shown in Figs. 2 (a) and (b).

Because leaf senescence was weak during the survey, it makes difficult to estimate the LLS on the saplings of parental species, therefore we estimated the T₅₀ which indicates how much the plant loses 50% of its leaves. In our case, the inter specific diversity of T₅₀ could be evaluated only on the first cohort of leaves followed, that of June 2010. T₅₀ was reached

between March and April 2011 for *Q. suber* and *Q. canariensis*. Leaves of *Q. canariensis*, showed a few days larger T50 than *Q. suber* (respectively 304 and 300 days; Figs. 2 (a) and (b)), while for *Q. afares* LLS did not exceed 304 days; it oscillated between 183 days for the cohorts of autumn and 304 days for spring cohorts (Fig. 2 (c)).

LLS of both *Q. suber* and *Q. canariensis* exceeded the duration of the experiment (17 months) on most cohorts but this was not the case for their hybrid (*Q. afares*) which had lost all leaves from all cohorts. Our survey showed that, in contrast to the adult trees grown in the field, which loose approximately 80% of their leaves at the beginning of the winter, the seedlings of *Q. canariensis* still kept their first leaves after two seasons of vegetation, which confirms the conclusions put forward by [23] for the same species. This was probably due to the milder winters in Tunis compared to the mountains. Also, the phenology of the seedlings of *Q. suber* and *Q. canariensis* showed a similar trend and the time taken by those species for the fall of leaves per cohort was prolonged for long time, whereas the hybrid showed very

short-lived leaves and a faster leaf fall. This offset may increase the competition between species at seedling stage. Leaf senescence in the hybrid could be the major cause of its smaller growth.

B. The Relative Growth Rates in Height and Diameter (RGR_H and RGR_{BD})

The comparison of RGR_H and RGR_{BD} did not reveal any significant differences among species for 2010 ($F = 0.57$, $P = 0.56$, and $F = 1.56$, $p = 0.19$, respectively). The range of values is between 0.53 and 0.60 $\text{cm cm}^{-1} \text{yr}^{-1}$ and between 0.53 and 0.66 $\text{mm mm}^{-1} \text{yr}^{-1}$, respectively for the height and the diameter. In 2011, a very significant species effect was detected ($F = 12.44$, $P < 0.0001$). Differences in RGR_H were observed between *Q. afares* and *Q. suber*, the latter showing lower values (0.53 $\text{cm cm}^{-1} \text{yr}^{-1}$) and between *Q. afares* and *Q. canariensis*, which had a higher value (0.60 $\text{cm cm}^{-1} \text{yr}^{-1}$). For RGR_{BD}, the differences were only recorded between *Q. afares* and *Q. canariensis*, the former displaying a higher value (0.66 $\text{mm mm}^{-1} \text{yr}^{-1}$) ($F = 6.21$, $P = 0.0003$) (Fig. 3).

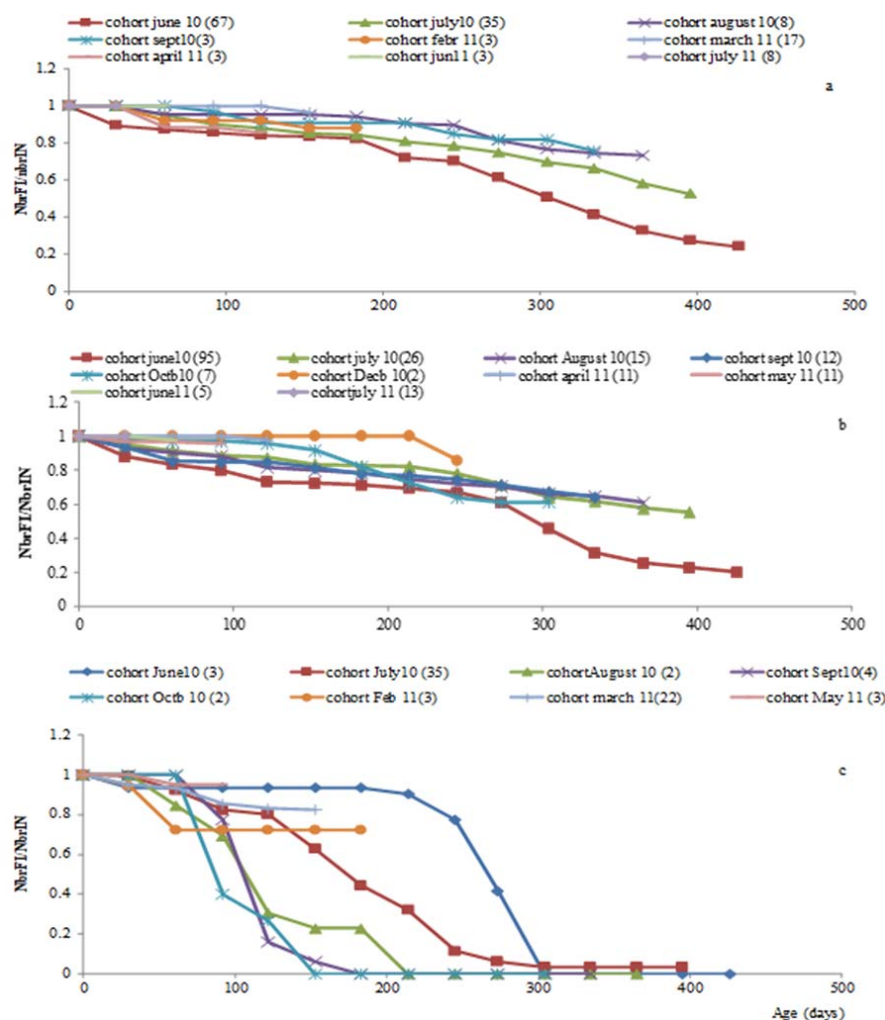


Fig. 2 Time course of the relative number of leaves of several cohorts on saplings of *Q. canariensis* (a), *Q. suber* (b) and *Q. afares* (c) grown in a nursery at Tunis, according to age (b) (the number of individuals by cohort is between brackets)

The results of this work demonstrate that the highest vertical growth corresponds to the bud bursts of the species, parental species displayed a significantly greater elongation than that of their hybrid.

The smaller growth of the saplings of *Q. afares* may be due to the fact that, in the nursery, growth was limited by the volume of the container for water and nutrient withdrawal, which does not reflect its growth under natural conditions, especially that the hybrid invests heavily in its root growth (personal observation).

Previous studies have shown that, besides external abiotic factors, seedling growth may vary according to some intrinsic characteristics of the species, such as seed mass, which

represents the quantity of reserves delivered to the plant during the beginning stages of its life [24], [25].

The study of [26] revealed that the growth and the morphology of seedlings of three species of oaks (*Q. canariensis*, *Q. pyrenaica*, and *Q. suber*) are affected by a wide number of resources and environmental conditions, such as light and mass of acorns.

According to our observations, the differences in growth patterns between species may reflect their local distribution patterns and seem to constitute a response of competition for light interception. Also, the low growth of the hybrid can be due to leaf fall and the dormancy state in winter.

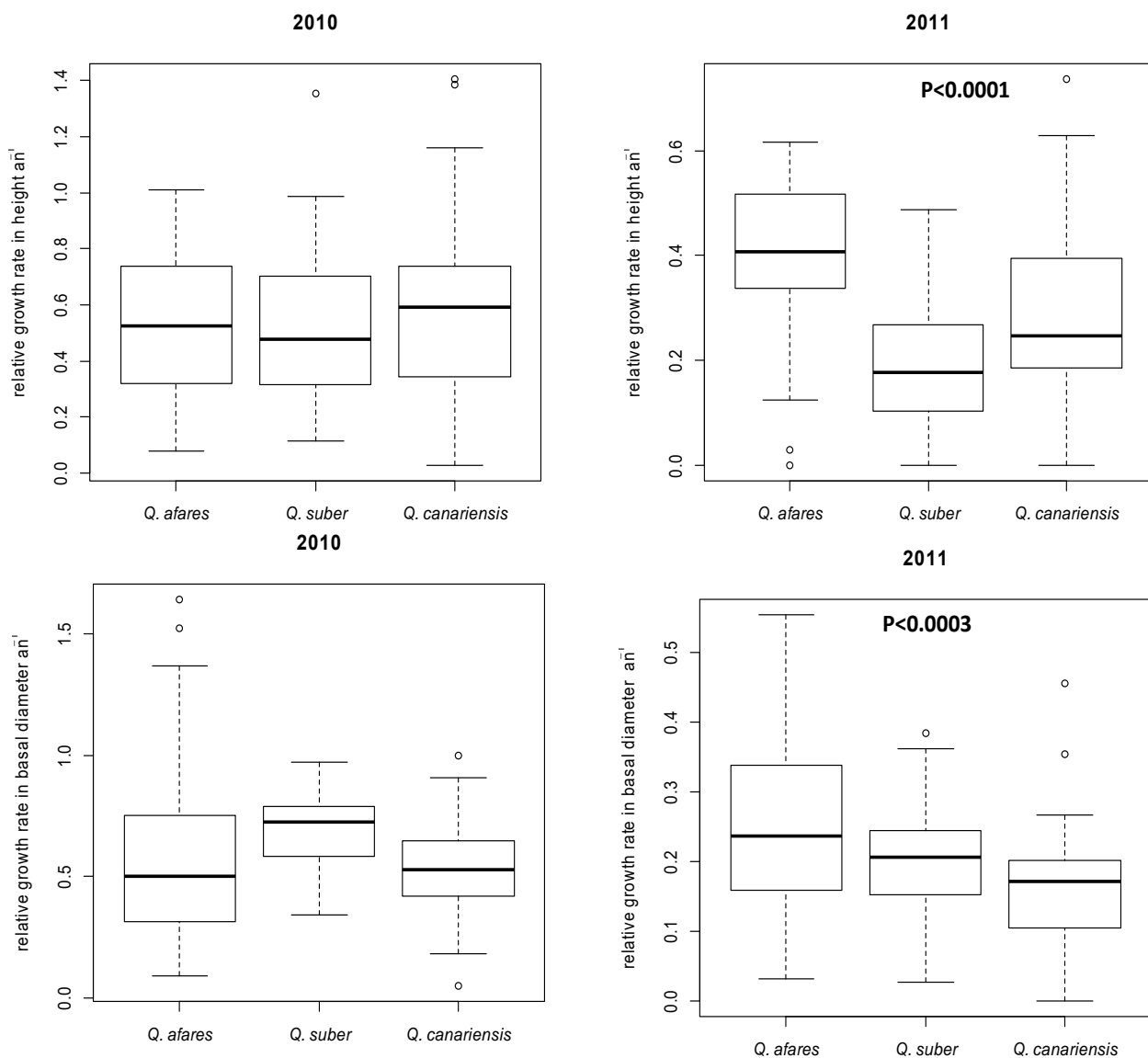


Fig. 3 Boxplots of the relative growth rates (RGRH) and diameters (RGRBD) of the shoot length on seedlings (the upper and lower edges of each box indicate the quartiles respectively 75% and 25%). The horizontal line in each box is the median and the vertical bars indicate the minimum and maximum values. Values beyond the range are represented by a circle, a value of $P < 0.05$ indicates a significant difference between the seedlings of the three species (one-factor ANOVA testing the effect of species)

IV. CONCLUSIONS

The results of phenological monitoring showed that LLS of the seedlings of *Q. suber* and *Q. canariensis* is longer than their hybrid (*Q. afares*). The shorter LLS of the hybrid at the seedling age concurs with its phenology in the field. But, for *Q. canariensis*, the long LLS contrasts with observations on adult's trees in the field, for which the phenology is strictly annual.

The growth of the seedlings during the follow-up occurs mainly in the spring and the autumn.

Parental species are the best performing for growth while their hybrid is less efficient.

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