Evaluation and comparison of phenological, morphological and ecophysiological features of 4 different species of Quercus, typical of tropical dry forests (Miraflor-Moropotente Park - Nicaragua)

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ABSTRACT

Phenological, morphological and ecophysiological variables were analyzed in winter (dry season) and in summer (wet season) for 4 species of Quercus sp. living in the Miraflor-Moropotente Natural Park, Estelí, Nicaragua. Each species has a slightly different altitudinal range of distribution in the studied area, and for each species two populations were chosen, located respectively in a low and in a high part of its range. All the parameters analyzed showed to vary significantly according to species, altitude and season, being the species the main discriminating factor. In particular, species that occupy lower altitudes display adaptation strategies that are typical of lower water regimes, such as more prolonged leaf fall and lower stomatal densities; inside single species such pattern are replicated, even if less markedly, for populations that occupy areas at lower altitude. It can be concluded that climatic factors, connected with altitude, significantly affect plant responses in the dry tropic, and that such responses vary among species as a result of different adaptation strategies. These strategies are at the basis of forest structure, composition and biodiversity, and subtend the species’ distribution patterns. Their better knowledge is a crucial tool for a more focused forest management in the frame of the ongoing climate change.

Keywords: dry tropic, gas exchange, phenology, Quercus sp.
Evaluación y comparación de las respuestas fenológicas, morfológicas y ecofisiológicas de 4 especies de Quercus típicas de los bosques del trópico seco (Paisaje Terrestre Protegido Miraflor-Moropotente – Nicaragua)

RESUMEN

Para este estudio se han analizado las variables fenológicas, morfológicas y ecofisiológicas de 4 especies de Quercus sp que se encuentran en el Paisaje Terrestre Protegido Miraflor de Miraflor-Moropotente (Esteli, Nicaragua), en invierno (estación seca) y en verano (estación húmeda). Cada especie presenta un diferente rango altitudinal de distribución, y para cada especie se han elegido dos poblaciones, ubicadas respectivamente en la zona baja y en la zona alta de dicha área. Todos los parámetros analizados varían significativamente entre especies, poblaciones y estaciones, con la especie como principal factor discriminante. En específico, las especies que ocupan altitudes menores presentan estrategias de adaptación típicas de regímenes hídricos más escasos, como la caída de hojas más prolongada en el tiempo y menores densidades estomáticas; este patrón se observa también entre poblaciones de altitudes diferentes dentro de cada especie, aunque menos marcado. Se puede concluir que los factores climáticos relacionados con la altitud afectan significativamente las respuestas de estos árboles típicos del trópico seco, y que dichas respuestas varían entre especies como resultado de diferentes estrategias de adaptación. Estas estrategias están a la base de la estructura, composición y biodiversidad de los bosques del trópico seco, e influencian los patrones de distribución de las especies. La mejor comprensión de dichas estrategias es una herramienta crucial para la gestión forestal de estos delicados ecosistemas en el marco del cambio climático en curso.

Palabras clave: fenología, intercambio de gases, Quercus sp., trópico seco.
INTRODUCTION

Tropical dry areas are mainly characterized by water stress due to the presence of a dry season with virtually no rains (Mooney et al., 1996), a total annual precipitation of less than 2000 mm (Cabrera et al., 2006) and temperatures between 17 and 35 °C (Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, 1998).

Due to the fertility of their soils, tropical dry areas have been in recent decades the centre of intensive agricultural development and transformation (Maas et al., 2005), nowadays making this ecosystem one of the most endangered of the neotropic (Miles et al., 2006).

Despite their environmental, social and economic importance, tropical dry areas historically have received less scientific attention than the humid ones (Prance, 2006), and are not as well protected as other tropical ecosystems (Pennington et al., 2006). Projections moreover demonstrate that in the future the extreme conditions of these ecosystems will be exacerbated by drivers of climate change, even if in some areas and in the first decades precipitations could even rise a bit (Hawkins et al., 2014); on the longer term it is predicted that the Central American regions will be affected by medium-long (4 - 6 months) drought periods, which will be 3 times more frequent than at present (Sheffield & Wood 2008).

The study of the adaptation strategies of key plant species of the dry tropic can be crucial in order to perform predictions about future shifts, and therefore the ecological consequences of the ongoing changes of climate (Hawkins et al., 2014).

Phenotypic plasticity is the process through which plants can acclimate to spatial and temporal variations of the environmental drivers (Savé et al., 1999) and of resource availability (Endara & Coley, 2011). Such phenomenon involves their water, nutrient and photosynthetic dynamics, and is expressed at various levels in all plant organs, both through short-term physiological responses and long-term physiological, structural and morphological modifications (Chaves et al., 2003). Moreover, within a same genus, different species can often display different adaptations which reflect their spatial distribution in nature (Zuccarini et al., 2015).

Phenology and the morphological and ecophysiological leaf characteristics, which are indicators of photosynthetic capacity, can be valid indicators of plant acclimation to the environment (Chaves et al. 2016; Grassein et al. 2010), due also to the relative simplicity of their measurements.

The genus Quercus is very representative of tropical dry forests (Flora de Nicaragua, 2007), and 12 species of *Quercus* have been reported in Nicaragua, being very common and usually dominating in pine-oak woods of the Central North Region, where they occupy a range of elevations approximately spanning from 700 to 1400 m (Almeda, 1995).

The Natural Reserve of Miraflor-Moropotente (13° 3’ 22” and 13° 7’ 30” N; 86° 29’ 15” and 86° 29’ 50” O; 874 mm average annual precipitation; 21.4 °C average temperature; 70 % relative humidity) has a high importance in agroforestry (Casasola et al., 2001) and is home to four species of Quercus, presented here following the elevational gradient of their spatial distribution (Flora de Nicaragua, 2007):

*Q. segoviensis*, the most abundant species, dominant in pine-oak woods, lives between 650 and 1400 m a.s.l., and here is observed between 794 and 1321 m a.s.l.;

*Q. sapotifolia* spans between 700 and 1500 m a.s.l., and can be found here between 787 and 1330 m a.s.l.;

*Q. cortesii*’s altitudinal range is 1200-1500 m a.s.l.; and in the Park can be found until 1400 m a.s.l.);

*Q. insignis*’ range is also 1200-1550 m a.s.l., and in the Park has been observed up to 1430 m a.s.l.
Aim of this work was to compare the adaptation strategies of these four different species of Quercus, typical of the dry tropic, to different levels of water availability associated to different seasons and different altitude, comparison achieved through the study of their phenological, morphological and ecophysiological responses.

**MATERIALS AND METHODS**

4 species of Quercus, living in the dry tropical part of Nicaragua, were analyzed for this study.

These species have altitudinal ranges that are largely overlapping, but are distributed according to a natural gradient that is, from the lowest to the highest: Q.segoviensis, Q.sapotifolia, Q.cortesii and Q.insignis.

Two stations for each species were studied, representing respectively a low and a high level inside the altitudinal range of the species itself: Naranjita (787 m a.s.l.) and El Robledal (1270 m a.s.l.) for Q.segoviensis; Naranjita (787 m a.s.l.) and El Matapalo (1321 m a.s.l.) for Q.sapotifolia; El Robledal (1270 m a.s.l.) and Zacaton (1405 m a.s.l.) for Q.cortesii; Las Vueltas (1327 m a.s.l.) and Zacaton 2 (1431 m a.s.l.) for Q.insignis.

Weather conditions were obtain from El Limon Experimental Station (Ruiz et al. 2012).

For each altitudinal station, three experimental plots of 1000 m² with similar orientation and edaphoclimatic conditions were selected.

Phenological observations were performed on two crossed transects in each plot. Ten trees of similar size and age were chosen per transect, giving a total of 20 trees, replicated three times, per field location.

Morphological and ecophysiological measurements were performed on three random individuals per plot, of similar size and age. 3 leaves per individual were selected, for each measurement, from an intermediate height in the canopy, which showed similar characteristics of age.

Phenological, morphological and ecophysiological parameters were analyzed.

- **Phenology**: Leaf appearance; Leaf fall; Flowering; Fruit appearance; Fruit fall
- **Morphology**: n° of stomata; Leaf specific Weight (SLW); Fractal Index (FI)
- **Ecophysiology**: Stomatal conductance; Cuticular transpiration rate (TRc); Relative water content (RWC)

Observations and measurements were taken in winter (dry season) and in summer (wet season) of the same year, apart from the phenological observations that were performed monthly along the whole year.

Number of stomata was counted only in winter, and stomatal conductance was measured only in summer.

The phenological observations were taken monthly from January to December on the selected trees according to the Fournier (1974) method.

Stomatal density was measured according to Savé et al. (2000). Leaves were first dehaired by pressing adhesive tape to the leaf surface and gently removing it. Then a small drop of contact glue (Super-Glue, Loctite) was spread on a slide and the leaf was placed on it, dehaired side face down, and pressed during few seconds. The leaf was then carefully removed from the slide, leaving its epidermal prints on it. The stomata left on the epidermal print were viewed and counted with a light microscope (Carl Zeiss, Jena, Germany).

Foliar area and perimeter were determined from computer imaging of harvested leaves (Image Tool 3.0), and used to calculate Specific Leaf Weight (SLW) (gDW / m-2leaf) and Leaf Fractal Index (Foliar Perimeter / Foliar area-1) (Serrano et al. 2005, Vaughn et al. 2011).
Stomatal conductance was measured on three random individuals per plot on 3 different leaves, selected from an intermediate height in the canopy, which showed similar characteristics of age, health status, and orientation. Measurements were performed with a leaf porometer (DECAGON®) during the hours of maximum solar irradiation (10:00 a.m. to 2:00 p.m.).

After the field measurements leaves were cut and immediately weighted (FW). Then they were placed in containers filled with distilled water and kept in darkness at 2-4°C during 24-48 hours to reach maximum turgor (TW). Leaves were then left out of water during 60 minutes to allow stomata to open, and cuticular transpiration rate was measured gravimetrically along a four hour period (one measurement every 30 minutes) (Vaughn et al. 2011).

Finally leaves were dried in forced draft oven at 75°C for 3 days to measure their dry weight (DW).

The formula used to calculate cuticular transpiration rate was:

\[ TR_c = \frac{(FW_i - FW_p)}{(DW \times T)} \]

where:
- FWi: leaf initial fresh weight
- FWp: leaf present fresh weight
- DW: leaf dry weight
- T: time

Analysis of Variance was performed for each morphological and ecophysiological parameter among the different species, stations and seasons; the significance level was placed for all analyses at p≤ 0.05. Statistical analyses were performed with GraphPad Prism 7.

RESULTS

The differences among replicated leaves of the same tree and among replicated trees of the same plot are not significant, so trees and plots have been treated as homogeneous groups of observations.

**Phenology**

Phenological data were consistent among altitudes, and showed marked differences among species (Fig.1). All of them lost leaves during the dry season (February-April), but while Q.sapotifolia, Q.cortesii and Q.insignis lost only the 25%, Q.segoziensis totally lost its leaves, which started to reappear from May.

Moreover, while Q.segoziensis and Q.sapotifolia lost leaves during three months (from February to April), leaf fall for Q.cortesii and Q.insignis was concentrated only in February.

**Flowering** was concentrated, for all species, at the end of dry season (March and April), but fruit appearance showed marked differences between the lower altitude species (Q.segoziensis and Q.sapotifolia) and the higher altitude ones (Q.cortesii and Q.insignis). In fact the former ones produced fruits only until June, while the latter ones went on uninterruptedly until the end of the year.

A similar interspecific pattern is displayed, as a consequence, by fruit fall: while Q.segoziensis and Q.sapotifolia lost their fruits only between June and July, Q.cortesii and Q.insignis showed continuous fruit fall from July-August until December.

**Morphology and ecophysiology**

The number of stomata varies very highly significantly (P<0.0001) according to the species, and highly significantly (p<0.001) according to the altitude (Fig.2a).

All the investigated species show a higher number of stomata in their higher altitude population, except from Q.insignis which shows the opposite pattern.
Q. segoviensis and Q. insignis show the highest average number of stomata per leaf, while Q. sapotifolia and Q. cortesii show lower and similar stomatal density.

The variation of **Specific Leaf Weight** is very highly correlated (p<0.0001) with species and season, and correlated (p<0.01) with altitude (Fig.2b). Winter values tend to be higher than the summer ones; Q. sapotifolia on average displays the highest values, while Q. insignis the lowest ones.

In most cases, the high altitude population of the same specie have a higher SLW than the low altitude one.

**Leaf Fractal Index** shows a very high variability among species (p<0.0001), with Q. sapotifolia displaying again the highest values and with a similar interspecific pattern as the one of SLW, but has no significant differences between seasons and stations (Fig.2c).

**Stomatal conductance** varies quite highly inside every single species and station, but significate differences between species and altitudes can be detected (Fig.2d). Higher altitude populations always show higher average values, while among species Q. cortesii shows the highest values and Q. insignis the lowest ones.

The values of **cuticular transpiration** are always slightly lower at lower altitudes (p<0.1), and despite varying in a very highly significant way among species (p<0.0001), there are no significant variations between seasons (Fig.2e). Q. insignis has the highest average values, while Q. segoviensis and Q. sapotifolia the lowest ones.

**DISCUSSION**

The main observed differences, for the studied parameters, are among species, followed by the altitude and finally by seasons.

The significant differences among species for phenological, morphological and ecophysiological parameters reflect how different species develop and show differential strategies to cope with the environmental drivers typical of their areas of distribution, which can change significantly according to altitude and topography (Méndez-Toribio et al., 2016). This interspecific variability has been observed by several authors in different geographical contexts (i.e. Scarano et al. 2005; Zuccarini et al. 2015), and is at the basis of the spatial patterns of distribution of tree species.

It is noteworthy that the species distributed at lower altitudes (Q. segoviensis and Q. sapotifolia) lose their leaves during a longer time span (three months instead of one), with Q. segoviensis losing the 100% of them: this indicates a phenological adaptation to conditions of lower water availability associated to the lower altitudes (Wright & Cornero, 1990). Moreover, this is reinforced by the fact that Q. segoviensis and Q. sapotifolia have a shorter period of fruit production (and consequently of fruit fall) along the year, showing a lower energetic investment and a more concentrated reproduction period.

A similar pattern to the above mentioned one can be seen in the morphological and physiological parameters. Species and populations of higher altitude, with the exception of Q. segoviensis, have higher stomatal densities, demonstrating a stronger investment in gas exchange which is allowed by the higher water availability. Ashton & Berlyn (1994) already observed, in Quercus sp., how species with higher drought tolerance displayed lower stomatal densities. This fact is also well reflected by the patterns of stomatal conductance among species and altitudes.

SLW variation also reflects the climatic conditions: plants that cope with lower water availability, or at least with longer periods of drought along the year, tend to develop thicker and harder leaves (Laureano et al., 2008). This is because a reduction of the relative
surface area means a reduction of the ways to lose water, even if for some species an opposite pattern has been observed (Aspelmeyer & Leuschner, 2006).

The low differences in fractal index between seasons and stations are probably due to the relatively high variability inside single populations, which covers all significant differences except the interspecific one, which is very high.

Cuticular transpiration shows a typical response related to altitude, so at lower mountain levels, longer growth period and higher drought stress is measured, which promoted great cuticle development respect higher altitude levels (Savé et al 1999), but these differences are not statistically relevant.

What can be inferred is that in these areas the dry season is not sufficiently long as to allow the development of a thicker and less permeable cuticle in the species and populations that cope with low water availability (see Yeats & Rose, 2013), also due to the high leaf turnover between seasons.

From the observed data we can conclude that climate factors (especially water availability) and altitude strongly influence forest structure, composition and diversity, through the differential adaptation strategies put in action by different tree species of montane oak forest communities in the dry tropic.

These differential strategies involve their phenology, morphology and ecophysiology, and are at the basis of their spatial distribution in nature.

The better knowledge of their responses to climate is the key to understand their present distribution and their future behaviour towards drivers of climate change, and the distributional shifts that will consequently occur, allowing a focused forest management aimed at preserving the equilibrium among land use and forest regeneration.

Also, present results can contribute to develop REDD policies (Ruiz et al 2016) and permit to develop mitigations strategies, using forest as a stable carbon sink.

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Fig.1: Phenological records of the observed species during the year of observations. The coloured boxes represent positive records.
Fig. 2: Morphological and ecophysiological features of the observed species at different seasons and altitudes. 

a: n. of stomata; b: Specific Leaf Weight; c: Fractal Index; d: Stomatal Conductance; e: Cuticular Transpiration.