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Physiological aspects of natural regeneration in coppiced forest dominated by *Quercus frainetto* Ten. and *Quercus cerris* L.

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Abstract

Comparison study of some physiological parameters of natural generative regeneration of *Quercus frainetto* Ten. (QF) and *Quercus cerris* L. (QC) was conducted *in situ* in an oak-dominated old coppiced forest. Gas exchange levels and sapling leaf pigment content were assessed simultaneously under the canopy (C-site) and in a gap (G-site) open in the stand two years before measurement. Differences in maximum photosynthetic capacity (A_{max}) and dark respiration rate (R_d), determined by light-response regressions were compared to evaluate leaves' carbon balance. Transpiration rate (E) and water use efficiency (WUE) were analyzed to compare changes in water regimes. Total chlorophyll content (TCC) and normalized difference vegetation index (NDVI) were monitored on the same plants. Species-specific responses to changed micro-condition after a canopy opening were observed. Both elements of the carbon balance (A_{max} and R_d) change much more substantially in QF leaves compared to QC in G-site. At the same time, the QF has a greater difference of E in the G-site compared to the C-site than in the QC. Such leads to a flattening of the WUE between two species at the G-site, in a background of a higher WUE of QF in the C-site. However, QF has similar, although not higher, TCC levels as in QC, as well as NDVI in G-site, compared to C-site.

Key words: natural regeneration, photosynthetic light response, physiological adaptation, water use efficiency.

Introduction

In recent years a broad and complex study of oak-dominated coppiced forests in Bulgaria has started. The process was driven by the goals set by the national meeting for managing of oak coppiced forests in Bulgaria (Kostov 2016). *Quercus frainetto* Ten. (QF) and *Q. cerris* L. (QC) have a determinant role in the Bulgarian oak coppiced forests (Kostov et al. 2017). In West Bulgaria, they often take part in mixed coppiced forests with the dominance of one or the other (Kostov et al. 2019). Although from different subgenera and sections (subg. *Quercus,* sect. *Quercus* and subg. *Cerris*, sect. *Cerris* respectively) of the genus *Quercus* (Hipp et al. 2019), according to the European atlas of forest tree species (de Rigo et al. 2016,

Mauri et al. 2016), *Quercus frainetto* Ten. and *Q. cerris* L. prefer similar ecological conditions with wider range for the second one.

As Kimmins (1997) says, 'Foresters will have to become much more familiar with autecology and ecophysiology of the plant communities they are managing if natural regeneration is to be accomplished successfully'. The main goals of the plant physiological studies in forest science may differ from an explanation of seasonal or age-related differences in some of the internal processes in the tree plants (Wolkerstorfer et al. 2011) to exploring the mechanisms for overcoming stress periods (Pietras et al. 2016; Stojanović et al. 2016 2017) or disturbances (Anev and Marinova 2021).

The present study aimed to compare Hungarian oak (*Quercus frainetto*) and Turkey oak (*Quercus cerris*) seedlings in adaptation to increased light intensity by comparing light curves of photosynthesis, transpiration rate and water-use efficiency (WUE) and total chlorophyll content. We also studied normalized difference vegetation index (NDVI) in relation to different microclimatic conditions, as a result of anthropogenic activity (gaps-opening phase during implementation of shelterwood with gaps regeneration system).

Materials and Methods

Site description

This *in situ* experiment was conducted in a mixed *Q. frainetto* (QF) and *Q. cerris* (QC) coppiced stand, which is a part of State Forestry Enterprise Elin Pelin and situated about 30 km east of Sofia in West Bulgaria. The site and stand characteristics are given in tables 1 and 2, respectively.

Table 1. Site description.

Characteristic	Value
Altitude, m a.s.l.	700
Latitude	42°35'38.99″
Longitude	23°36'34.60″
Slope, °	5
Aspect	North
Mean annual air temperature, °C	8.0
Total precipitation, mm·yr^{-1}	574

Table 2. Stand description.

Characteristic	*Q. frainetto*	*Q. cerris*
Age, years	60	60
Basal area, m²·ha^{-1}	9.2	13.1
Density, N·ha^{-1}	216	208
Mean diameter (DBH), cm	24	28
Mean height, m	15	18
V, m³·ha^{-1}	68	108

The regeneration on the site was abundant with more than 196,000 ha^{-1} (~29 %) seedlings for QF and more than 473,000 ha^{-1} (~71 %) for QC, mostly about 0.30 m up to 0.50 m of height and mostly about 5–6 up to 12–15-years-old, regularly distributed all over the area of the stand.

During the winter of 2017, a gap was opened with an area of 1252 m^2 within the experimental stand. This study was conducted in June 2019 when the saplings in the gap had developed leaf buds laid under the new light conditions.

Plant material

Three different natural regenerated sample seedlings from QF and QC, both in the gap and under the canopy, or total of 12 seedlings were used in this study. For the gas-exchange and pigment concentration measurements, a third fully developed leaf from the top of the experimental sap-

Fig. 1. Experimental leaves, taken from saplings: a) in the gap, and b) under the canopy.

lings was chosen (Fig. 1).

Gas-exchange and pigment concentration

Gas-exchange measurements were conducted with a portable photosynthesis system Li-6400 (Li-COR Inc., Lincoln, NE, USA), equipped with shielded thermocouples, a red light-emitting diode light source (6400-02), and a quantum PAR sensor (LI-190) in the chamber. Before measurements, the Li6400 was calibrated according to the manufacturer's recommendations (LiCor 2012). Following standard protocol (Evans and Santiago 2014), light-response curves for each leaf were recorded on a typical sunny day, between 09:00–12:00 at ambient levels of environmental factors: with CO_2-concentration at about 395 μmol-CO_2·mol^{-1}, leaf temperature 23.5 °C, and 43.3 % relative humidity and using eight levels of photosynthetic photon flux density (2000, 1000, 500, 250, 100, 50, 25 and 0 μmol·m^{-2}·s^{-1}). Photosynthetic light responses were individually analysed with the non-rectangular hyperbolic model (Prioul and Chartier 1977, Leverenz and Jarvis 1979, Marshall and Biscoe 1980) by formula (1):

$$An = \frac{\alpha \cdot \mathrm{PPFD} + A_{\max} - \sqrt{\left(\alpha \cdot \mathrm{PPFD} + A_{\max}\right)^2 - 4 \cdot \theta \cdot \alpha \cdot \mathrm{PPFD} \cdot A_{\max}}}{2 \cdot \theta} - R_d, \qquad (1)$$

where: *An* is the photosynthetic rate, PPFD is the incident photosynthetic photon flux density, *α* is the maximum apparent quantum yield (the initial slope of the curve), *θ* is the convexity of the light response curve, and A_{max} is the light-saturated gross photosynthetic rate, R_d is the dark respiration rate.

All regression lines were fitted to the data by applying the least-squares approach of the Microsoft Excel Solver routine using the Newton algorithm (Office 2016, Microsoft, Redmond, WA, USA).

Total chlorophyll content (TCC, g·m^{-2}) and normalized difference vegetation index (NDVI) were measured with a portable chlorophyll meter atLEAF+ (FT Green LLC, Wilmington, DE, USA) and PlantPen NDVI-300 (PSI Corporation, Czech Republic) respectively, using two readings per leaf (one on each of the left and right parts of the leaf). The atLEAF+ and

Plant-Pen NDVI-300 meters measure leaf absorptance or reflectance difference between red and near infra-red lights, and their measurements strongly correlated to the total chlorophyll and water content of leaf (Muraoka et al. 2012, Zhu et al. 2012, Novichonok et al. 2016).

Two-way ANOVA (Factor A: Species and Factor B: Conditions) with Tukey's multiple comparison test was used for statistical evaluation of experimental variants.

Results

Light curves of photosynthesis and CO_2-balance

Under the canopy, QF is more strongly suppressed, and its photosynthesis quickly reaches saturation. QC takes advantage of direct incident light more adequately and, at PPFD above 750 μmol(γ)·m^{-2}·s^{-1}, realizes higher photosynthesis than QF (Fig. 2a).

In the gap, the light curve of photosynthesis of QC leaves does not differ in comparison to such under the canopy. However, the photosynthetic light curve of QF was changing significantly, and already in a much more comprehensive range of PPFD levels, QF is competitive with QC (Fig. 2b).

In confirmation of the above, the parameters of the light curve regressions referring to the balance of CO_2 change specifically in the two species, depending on the condition – under the canopy or in the gap (Fig. 3).

In the gap, A_{max} is only 1.18 times higher for QC, while for QF, this difference is significantly higher (1.58 times). A larger increase in A_{max} may signal a faster adaptation to the illumination of QF than QC. At the same time, the dark respiration rate (R_d) is significantly higher within the gap only in QF (2.34 times), while QC varies

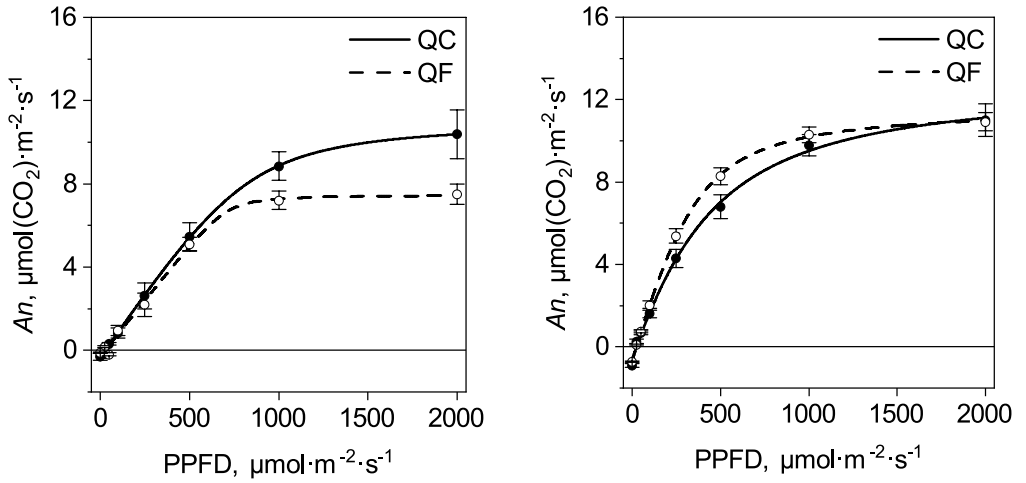

Fig. 2. Photosynthetic light-responses of *Quercus cerris* (QC) and *Quercus frainetto* (QF) under the canopy (a) and within the gap (b).

Note: Arithmetic mean values (n = 3) of the measured photosynthesis at different PPFD levels are shown with dots and their standard errors – with whiskers. The non-linear regressions according to eq. (1) are shown with lines.

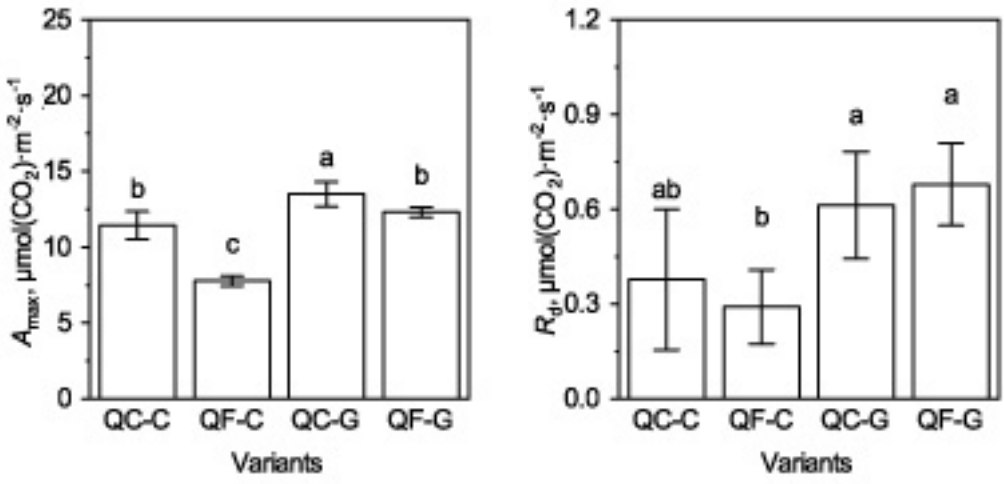

Fig. 3. Maximum photosynthetic rate (A_{max}) (left) and dark respiration rate (R_d) (right) of QC and QF within the gap (G) and under the canopy (C).

Note: Data represent the regression coefficient ±SE. Two-way ANOVA (Factor A: Species and Factor B: Conditions) (Tukey test) is applied to estimate the difference between all the variants. Different letters denote statistically significant differences (p-value < 0.05).

insignificantly between the canopy and gap, which gives an advantage in the carbon balance of QC compared to QF.

Water regime

Under the canopy, QF transpires with lower (1.66 times) intensity than QC, but within the gap, increased transpiration rate (2.7 times) much more than QC (1.51 times) and thus gained its water consumption with that of the QC (Fig. 4).

Thus, despite the more efficient use of water under the canopy of QF, the WUE in the gap is equalized between species and significantly lower than under the canopy.

Total chlorophyll content and normalized difference vegetation index (NDVI)

TCC was higher only in the leaves of QC in gap compared to the canopy, which can be interpreted as the formation of sun ecotype leaves characterized by thicker leaf lamella and, therefore, more chlorophyll per unit leaf area. However, for the QF leaves did not observe a similar difference in TCC under the canopy and in the gap (Fig. 5).

The leaves of both species retain high levels of NDVI (Gamon et al. 1995), despite specific variations in their chlorophyll content and water regime. The lack of significant change in NDVI can be interpreted as evidence of successful adaptation to gap conditions in both species.

Discussion

Turkey oak is a fast-growing tree with good adaptability (Savill 2013). It dominates in the regeneration and even at the same age and with equal participation in the stand density, its mean diameter and

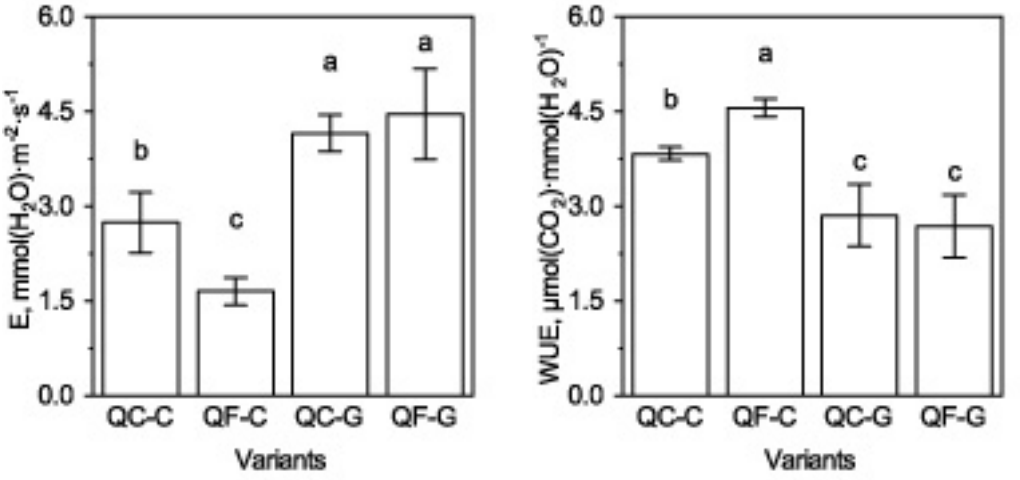

Fig. 4. Transpiration rate (E) (left) and water-use efficiency (WUE) (right) of QC and QF within the gap (G) and under the canopy (C).

Note: Data represent the mean values (n = 3) ±SE. Two-way ANOVA (Factor A: Species and Factor B: Conditions) (Tukey test) is applied to estimate the difference between all the variants. Different letters denote statistically significant differences (p-value < 0.05).

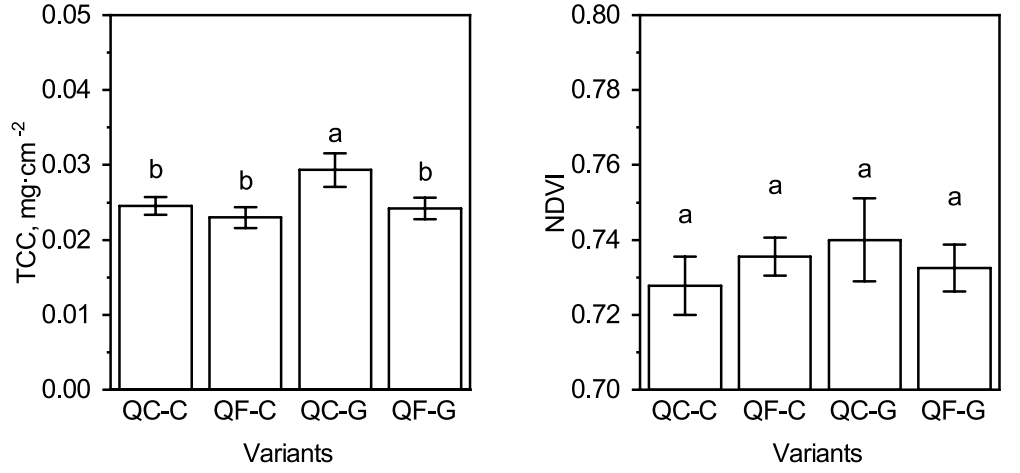

Fig. 5. Total chlorophyll content (TCC) (left) and normalized difference vegetation index (NDVI) (right).

Note: Data represent the mean values (n = 6) ±SE. Two-way ANOVA (Factor A: Species and Factor B: Conditions) (Tukey test) is applied to estimate the difference between all the variants. Different letters denote statistically significant differences (p-value < 0.05).

height and the basal area and stock volume per ha are greater. Since the site characteristics are favourable for both species, we can suggest this dominance of QC is a result from the physiological processes within the trees.

Condition-derived forms (Kimmins 1997) of light-response curves were

concluded for both species, considered light-dependent, showing suppression in their seedlings under the canopy with greater scale for QF. Yet, species-specific features were observed in their responses within the opening. Both groups in the gap reach high levels of net photosynthesis but also high levels of dark respiration and the saplings under the canopy stand contrary to them. The results of net photosynthesis within the gap are similar to those of older trees from the same species during the same season described by Wolkerstorfer et al. (2011), but those under the canopy show again an advantage of QC. Clearly, under the canopy, both species develop lower productive leaves with a lower response to the increasing light intensity, but this is more pronounced in QF. The higher light response under canopy conditions shows better light-dependent competitiveness of *Q. cerris* and higher light-related stress in *Q. frainetto* under the canopy.

CO_2 exchange shows higher capabilities of QC to include carbon into organic compounds under the canopy. It reaches relatively high values under both conditions, showing almost no sign of suppression under the canopy. QF manages to fix CO_2 at a slightly higher rate than QC in the gap, but under the canopy, it falls behind, showing the lowest maximal rate of photosynthesis. With the small amount of CO_2 released with respiration, we can say that CO_2 exchange in QF-C is highly suppressed.

Both species showed higher levels of transpiration than older trees during the same season (Wolkerstorfer et al. 2011), which can be referred to the higher rate of most physiological processes in younger individuals. Within the gap, QF increases its rate of transpiration on a greater scale than QC and decreases water use efficiency dramatically close to the QC WUE. The similar E and WUE in the gap show no signs of water stress. This could be another sign of light-dependent stress in QF under the canopy. QC remains more active under the canopy, so its transpiration rate remains relatively high.

All studied physiological aspects of the regeneration in this study show suppression of QF under the canopy and the ability of QC to remain vital under some level of shading, in conscience to Popovic et al. (1997). In the gap the species are with comparable performance with slightly earlier response in QF.

The NDVI index for all the groups is relatively high, showing that the plants chosen for the study are healthy, without a sign of drought-related stress. They also have close chlorophyll concentration levels, although QC-G has the greatest. The latest probably shows the mechanism for better light-dependent performance in this case, and it is higher chlorophyll concentration.

The uses of the two species differ, with the preference of the Hungarian oak for timber (Mauri 2016) and the Turkey oak primarily for fuel wood (Savill 2013). There should be chosen a primary aim in front of any mixed natural stand or plantation from these species that is going to be managed. The intensity and repetition of thinning during early stages of stand development can be corrected by this aim.

Conclusions

The study confirmed that both species are shade-intolerant, more pronounced for QF. The closed canopy suppresses both species but QF more. The opening of gaps in the canopy reflects in greater scale the QF but favours both species. The better

performance for QC under some levels of shading makes it more competitive from both, so that we can expect its dominance during the following stages of stand development for mixed *Q. frainetto – Q. cerris* stands without further selective thinning. The site conditions are favourable for both species, so the main question when managing naturally regenerated stands or establishing plantations on such conditions from these species should be the aim of the stand or do we want to produce fuel wood or timber.

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