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Ecophysiological behavior of Mediterranean woody species under summer drought

Comportamiento ecofisiológico de las especies leñosas mediterráneas ante la sequía estival

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SUMMARY

This paper investigated the effect of summer drought conditions on physiological and biochemical parameters in *Pinus nigra* subsp. *pallasiana*, *Pinus brutia*, *Quercus infectoria* and *Crataegus monogyna* under field conditions and determined seasonal changes in those parameters. The study focused on soil water content, soil temperature, midday water potential, proline, total soluble sugar and photosynthetic pigment (chlorophyll a, chlorophyll b, chlorophyll a+b and carotenoid) contents during the growing period. Seasonal changes in those parameters in *Pinus nigra* subsp. *pallasiana, Pinus brutia*, *Quercus infectoria* and *Crataegus monogyna* were determined under the same environmental conditions in the natural forest area of the western Mediterranean region. *P. nigra, P. brutia*, *Q. infectoria* and *C. monogyna* had similar seasonal changes in physiological responses, though they had different seasonal changes in biochemical responses in natural habitat. *Crataegus monogyna* had the lowest midday water potential at all sampling dates. In general, *Q. infectoria* had the highest photosynthetic pigment content, whereas *P. nigra* and *P. brutia* had the lowest. *P. nigra* and *P. brutia* (coniferous) had lower photosynthetic pigment content and higher midday water potential than those presented by *Q. infectoria* and *C. monogyna* (broad-leaved) during the short-term dry period. The results showed that *P. nigra* and *P. brutia* (coniferous) were more sensitive than *Q. infectoria* and *C. monogyna* (broad-leaved) during summer drought in the natural forest.

Key words: midday water potential, drought, woody species, proline, pigments.

RESUMEN

Este trabajo investigó el efecto de las condiciones de sequía estival sobre parámetros fisiológicos y bioquímicos en *Pinus nigra* subsp. *pallasiana*, *Pinus brutia*, *Quercus infectoria* y *Crataegus monogyna* en condiciones de campo y así determinar los cambios estacionales en dichos parámetros. El estudio se centró en el contenido de agua del suelo, su temperatura, el potencial hídrico a mediodía, la prolina, el azúcar soluble total y el contenido de pigmentos fotosintéticos (clorofila a, clorofila b, clorofila a+b y carotenoides) durante el periodo de crecimiento. Se determinaron los cambios estacionales de estos parámetros bajo las mismas condiciones ambientales en la zona de bosque natural de la región mediterránea occidental. *P. nigra*, *P. brutia*, *Q. infectoria* y *C. monogyna* tuvieron cambios estacionales similares en sus respuestas fisiológicas, aunque tuvieron diferentes cambios estacionales en las respuestas bioquímicas en el hábitat natural. *C. monogyna* tuvo el potencial hídrico más bajo a mediodía en todas las fechas de muestreo. En general, *Q. infectoria* tuvo el mayor contenido de pigmento fotosintético, mientras que *P. nigra* y *P. brutia* tuvieron el más bajo. *P. nigra* y *P. brutia* (coníferas) tuvieron un menor contenido de pigmento fotosintético y un mayor potencial hídrico al mediodía que *Q. infectoria* y *C. monogyna* (frondosas) durante el periodo seco de corta duración. Los resultados mostraron que *P. nigra* y *P. brutia* (coníferas) fueron más sensibles que *Q. infectoria* y *C. monogyna* (frondosas) durante la sequía estival en el bosque natural.

Palabras clave: potencial hídrico de mediodía, sequía, especies leñosas, prolina, pigmentos.

INTRODUCTION

Global warming has been a serious threat to forest ecosystems during the twenty-first century (Arend *et al.* 2011). Plants of the Mediterranean region are exposed to environmental stressors, especially in summer witnessing high midday sunlight and temperature, resulting in water depletion (Munné-Bosh and Peñuelas 2004). High temperature and water shortage in the drought-prone areas of the Mediterranean basins reduce the growth rates of tree species. It is crucial to understand how plants respond to drought stress caused by global warming (Nuche *et al.* 2014) because plants of the Mediterranean region are exposed to drought stress lasting several months in summer (Archibold 1995). Plants of the Mediterranean region develop adaptation mechanisms to survive long-term drought stress and water depletion (Baquedano and Castillo 2006). They develop many morphological, physiological, biochemical and molecular mechanisms to adapt to drought (Lei *et al.* 2006). The first of those mechanisms is stomatal closure as a response to water deficit to reduce transpiration and lower water potential (Martin-StPaul *et al.* 2017). Plants improve drought tolerance by reducing osmotic potential through solute accumulation (Puigdefábregas and Pugnaire 1999). Soluble sugars and proline are two solutes that play a crucial role in osmotic adjustment in plants (Hessini *et al.* 2009). Photosynthetic pigment content, which regulates energy absorption through chlorophyll and distributes excess energy through carotenoids, is another parameter that plays a key role in drought adaptation and survival in plants (Baquedano and Castillo 2006).

The red pine (*Pinus brutia* Ten*.,* 5.74 million ha) and the Anatolian black pine (*Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe*,* 4.35 million ha) are the two most common coniferous species in Turkey (OGM 2019). The red pine is generally found in areas with hot and dry summers and warm and rainy winters (Karatepe *et al.* 2014). The Anatolian black pine has high genetic diversity, and thus, a high adaptability to different climatic conditions, from humid, semi-humid to semi-arid climate. The Anatolian black pine is a xerophilic species distributed as mixed forest (red pine and scrub) in the transition zone between red pine and Anatolian black pine (Atalay and Efe 2010). Oaks (*Quercus* sp.) are distributed across 5.96 million ha in Turkey (OGM 2019). *Quercus infectoria* Olivier is a tree of semi-humid climates and withstands cold to some extent (Öztürk 2013). Hawthorn (*Crataegus* sp.) is ubiquitous in the cold and dry regions of Turkey, and many species are also drought-resistant (Gültekin 2007). Physiological and biochemical mechanisms play a key role in adaptation to drought conditions during summer. Drought is a serious problem in the Mediterranean region. We can better manage natural ecosystems if we know how different species in the Mediterranean region respond to water stress physiologically (Bombelli and Gratani 2003). This study had three objectives: It's a small font, it should be corrected. *(Pinus nigra* Arn. subsp. *pallasiana*, *Pinus brutia*, *Quercus infectoria)* and one shrub species' (*Crataegus monogyna* Jacq.) physiological and biochemical responses during summer drought, (2) to determine which species (coniferous and broadleaved) are more sensitive or tolerant to drought in the field, and (3) to identify the seasonal changes in soil water content, soil temperature, midday water potential, total soluble sugar content, proline content and photosynthetic pigment content. We hypothesized that summer drought affects woody species differently in their natural habitats.

METHODS

Site description and study species. The sample consisted of three species *Pinus nigra* subsp. *pallasiana*, *Pinus brutia*, *Quercus infectoria*, and one shrub species *Crataegus monogyna* located in the Aziziye/Burdur forest district in Turkey (37° 24' N; 30° 12' E; Altitude: 1,340 m; figure 1).

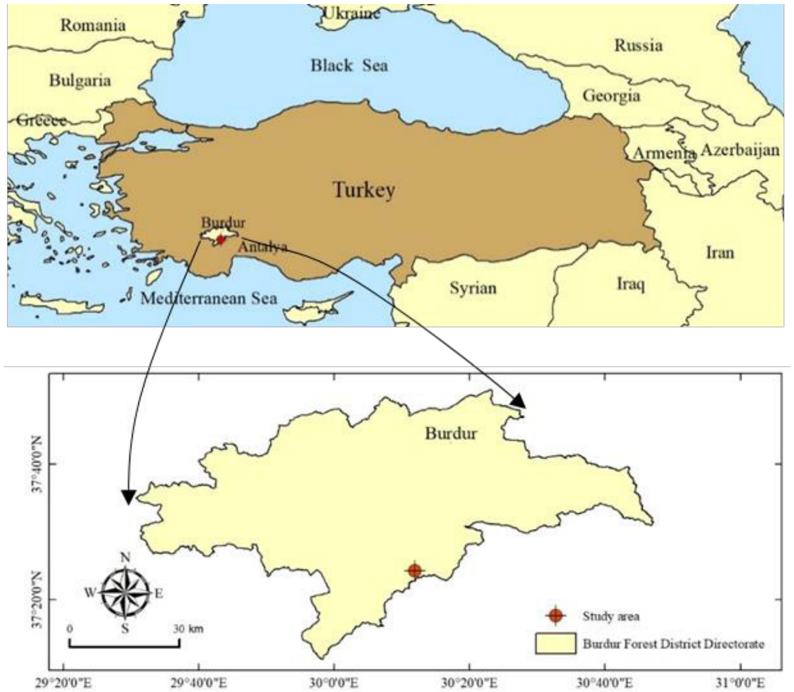

Figure 1. Location of study area.

Localización del área de estudio.

The experimental site was located on a claystone bedrock and had loamy sand soil. According to the long-term (2007-2016) climate data of Burdur Meteorology Station, the search site had annual total precipitation of 421 mm, average temperature of 13 °C and semi-arid climate according to Erinc climate index. In Burdur, December and January are humid, November, February, March and April are semi-humid, May is semi-arid, June and October are dry, and July, August and September are fully arid or desert type (Sarı 2009). A mini meteorological station (Watchdog Spectrum Technologies, Inc. USA) was installed near the research site in 2016 to record data at 30-min intervals to determine microclimatic conditions throughout the study. According to the climate data of the mini meteorological station, the research site had total precipitation from 10.10 mm (July) to 79.30 mm (May), average monthly temperature from 12.9 °C (May) to 23.5 °C (July), maximum temperature from 29.4 °C (May) to 38.3 °C (July), minimum temperature from 2.3 °C (May) to 12.2 °C (July), and average humidity from 64.1 % (May) to 38.1 % (July) throughout the study (May-September) (figures 2A; 2B; 2C; 2D; 2E). The highest average, maximum, minimum temperature and lowest relative humidity were recorded in July.

Sampling. The measurements were performed on a sample area of approximately 1,000 m^2 in a location close to the

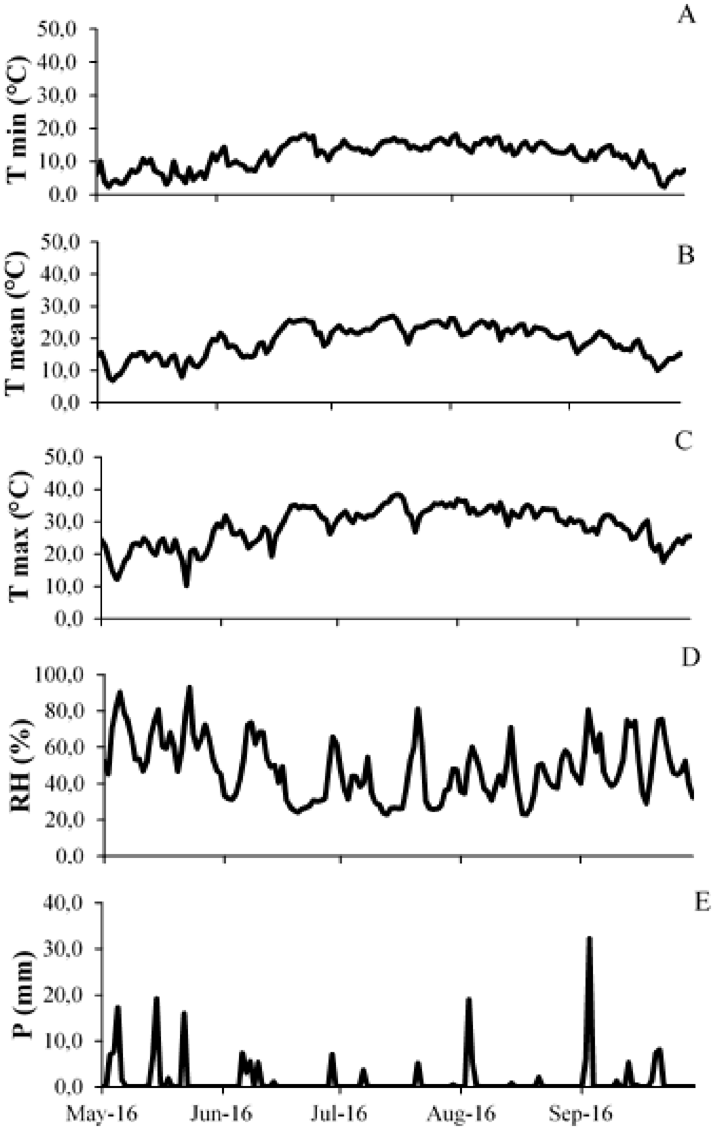

Figure 2. A) Daily minimum air temperature (Tmin). B) daily average air temperature (Tmean). C) daily maximum air temperature (Tmax). D) daily average relative humidity (RH) and E) daily total precipitation (P) during the study period.

A) Temperatura mínima diaria del aire (Tmin), B) Temperatura media diaria del aire (Tmean), C) Temperatura máxima diaria del aire (Tmax), D) Humedad relativa media diaria (HR) y E) Precipitación total diaria (P) durante el periodo de estudio.

middle of the stand. *P. nigra* had mean diameter at breast height, mean height and mean age of 11.3 cm, 5.7 m and 24 years, respectively. *P. brutia* had mean diameter at breast height, mean height and mean age of 11.8 cm, 6.4 m and 22 years, respectively. *Q. infectoria* had mean diameter at breast height, mean height and mean age of 5.8 cm, 2.6 m and 23 years, respectively. *C. monogyna* had a mean age of 24 years. Eight individuals from each species in the sample area were randomly selected, and shoot samples were collected from two-third of the trees/shrub and from the south-facing plot at five different dates (May, June, July, August, September) in 2016 to determine physiological (midday water potential) and biochemical characteristics (proline content, total soluble sugar content and photosynthetic pigment content). A biochemical analysis was determined on the 1- year-old needles of conifers and current-year leaves (fully expanded) of broad-leaved trees. Deciduous *Q. infectoria* and *C. monogyna* grew new leaves completely in mid-May.

Soil moisture and temperature. Soil water content and soil temperature were measured monthly between May and September at nine locations at a depth of 0-20 cm for homogeneous representation of the research site. Soil water content and soil temperature were measured using a gravimetric method and a digital thermometer (TP3001), respectively.

Water potential. Midday water potential was measured between 12:00 p.m. and 14:00 p.m., which was the period of time when water tension was the highest. Ψ_{md} was measured on one-year-old shoot samples from each species using a pressure chamber (Scholander *et al.* 1965; Model 1000, PMS Instruments Company, Corvallis, OR).

Soluble sugars and proline. Leaf and needle samples from each species were dried at 65 °C for 48 h and subsequently ground. Total soluble sugar content was measured using the method of Dubois *et al.* (1956). The samples were collected from the ground leaves and needles and were incubated for 24 h in 80 % ethanol. Afterward, 5 % phenol solution and H_2SO_4 were added to the samples, which were after measured using a spectrophotometer at a wavelength of 490 nm. Proline content was determined using the method of Bates *et al.* (1973). Three percent sulfosalicylic acid was added to 100 mg of dry samples, which were, next, filtered using a homogenate blue band filter paper. The filtrate was mixed with acid ninhydrin and glacial acetic acid, and afterwards, incubated at 100 °C for one h. Toluene was added to the cooled samples, which were then measured on the spectrophotometer at a wavelength of 520 nm.

Pigments. Photosynthetic pigment content (chlorophyll a, chlorophyll b, chlorophyll a+b and carotenoid) in fresh leaf and needle samples was determined using the method of Arnon (1949). The samples (0.1 g) were crushed to homogeneity in 10 mL of 80 % acetone solution in a mortar. The homogenized samples were measured on the spectrophotometer at wavelengths of 450, 645 and 663 nm.

Statistical analyses. The data were analyzed using the Statistical Package for Social Sciences (SPSS for Windows v 25.0) at a significance level of 0.05. A one-way analysis of variance (ANOVA) was used to determine significant ($P < 0.05$) differences in physiological and biochemical parameters measured at different sampling dates between the species. A variance analysis was also used to determine the effect of sampling dates on the physiological and biochemical parameters of the species. The Duncan's test was used to identify the source of difference. Sampling date, species and sampling date x species interaction were analyzed using a generalized linear model. A Pearson's correlation analysis was used to determine the correlation between the parameters.

RESULTS

Soil moisture and temperature. Sampling date affected soil water content and soil temperature ($P < 0.001$). Soil water content was the highest in May (21.8 %) and the lowest in July (11.1 %), August (11.2 %) and September (13.0 %). It gradually decreased towards July and remained relatively the same until September. Soil temperature was the highest in July (22.9 °C) and the lowest in May (14.7 °C) (figure 3).

Midday water potential. Species, sampling date and species x sampling date interaction affected midday water potential ($P < 0.001$; table 1). It was -2.21 MPa in *C. monogyna* and ranged from -1.60 to -1.83 MPa in *P. nigra*, *P. brutia* and *Q. infectoria* in May, which is a rainy month. It was almost the same in *P. nigra*, *P. brutia* and *Q. infectoria* in May and June, though it significantly decreased in July. The highest decrease in midday water potential in *C. monogyna* (-2.20 MPa) was recorded in July. All four species had the lowest midday water potential in July and August (figure 4). In July, *P. nigra*, *P. brutia*, *Q. infectoria* and *C. monogyna* had a midday water potential of -2.29 MPa, -2.45 MPa, -3.08 MPa and -4.23 MPa, respectively; while in August, they had a midday water potential of -2.15 MPa, -2.51 MPa, -3.10 MPa and -4.20 MPa, respectively. With rain in September, an increase was recorded in midday water potential, which almost reached the levels recorded for May. However, the increase in midday water potential was less in *P. nigra* than in *P. brutia*, *Q. infectoria,* and *C. monogyna*. There was a significant correlation between midday water potential, soil water content and soil temperature. In *P. nigra, P. brutia*, *Q. infectoria* and *C. monogyna* species, midday water potential was positively correlated with soil water content (respectively, R^2=0.527, R^2=0.371, R^2=0.365, R^2=0.365; $P < 0.001$) and negatively correlated with soil temperature (R^2=0.745, R^2=0.745, R^2=0.771, R^2=0.887; $P < 0.001$) (figure 5).

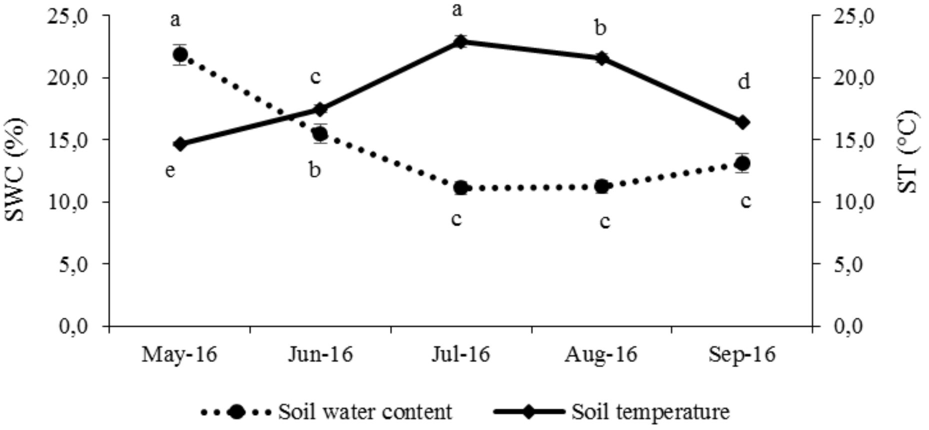

Figure 3. Seasonal changes in soil water content (SWC) and soil temperature (ST) in P. nigra, *P. brutia*, *Q. infectoria*, and *C. monogyna* (Lines indicate means ± SE)

Cambios estacionales en el contenido de agua del suelo (SWC) y la temperatura del suelo (ST) en *P. nigra*, *P. brutia*, *Q. infectoria* y *C. monogyna*.

Table 1. Analysis of general linear models of the effects of sampling date, species and species x sampling date interaction on midday water potential (Ψ_{md}), total soluble sugar content (TSS), proline content (PC), chlorophyll a (Chl_a), chlorophyll b (Chl_b), total chlorophyll (Chl_{a+b}), carotenoids (Car.).

Análisis de modelos lineales generalizados del efecto de la fecha de muestreo, la especie y la interacción especie x fecha de muestreo sobre el potencial hídrico de mediodía (Ψmd), el contenido total de azúcares solubles (SST), el contenido de prolina (PC), la clorofila a (Chla), la clorofila b (Chlb), la clorofila total (Chla+b) y los carotenoides (Car.).

Study variables	Factors		Interaction
	Species	Sampling date	Species x Sampling date
Df	3	4	12
Ψ_{md} (MPa)	**	**	**
TSS (mg g^{-1} DW)	**	**	**
PC (µmol g^{-1} DW)	*	**	**
Chl_a (mg g^{-1})	**	**	**
Chl_b (mg g^{-1})	**	**	**
Chl_{a+b} (mg g^{-1})	**	**	**
Car. (mg g^{-1})	**	**	**

*$P < 0.05$; **$P < 0.001$

The lowest midday water potential was recorded in *C. monogyna* in August. However, the lowest negative values were recorded in *P. nigra* ($P < 0.01$). Therefore, *P. nigra* had the highest midday water potential, followed by *P. brutia, Q. infectoria* and *C. monogyna* in August. *Pinus nigra* and *P. brutia* had similar midday water potential in July, while *P. nigra*, *P. brutia* and *Q. infectoria* had similar midday water potential in September, which was higher than that shown by *C. monogyna*.

Biochemical measurements. Both species and sampling date had a significant effect on total soluble sugar (table 1; $P < 0.001$). *Pinus brutia* had the highest total soluble sugar content, followed by *Q. infectoria*, *P. nigra* and *C. monogyna*. *Crataegus monogyna* had always the lowest total soluble sugar content, except in July. Moreover, midday water potential ($R^2 = 0.492$, $P < 0.001$) and soil water content ($R^2 = 0.395$, $P < 0.001$) were negatively correlated with total soluble sugar content only in *C. monogyna*. All species had similar total soluble sugar in July (figure 6). *Pinus nigra* had low total soluble sugar in May, June and July. It suddenly increased from August to September, when it became higher -in September- than in other species at all sampling dates. After the fall in July, *P. nigra* accumula-

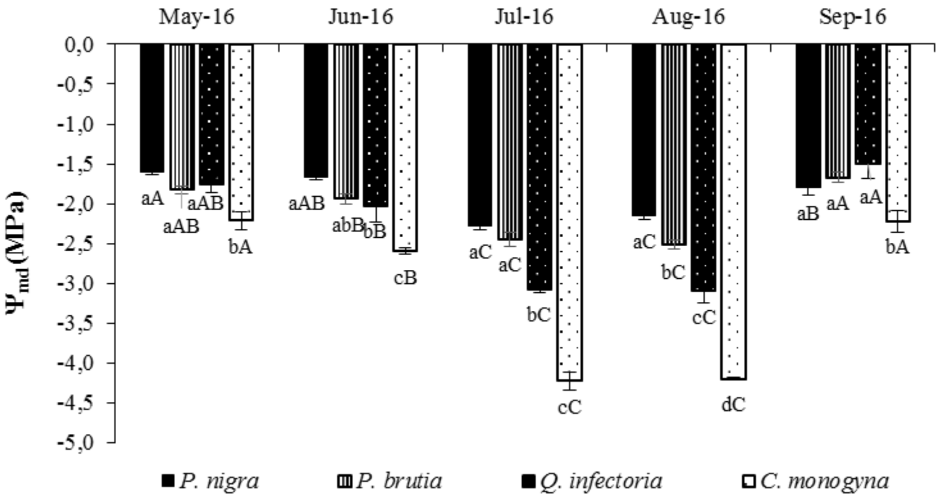

Figure 4. Seasonal changes in midday water potential (Ψ_{md}) in *P. nigra, P. brutia*, *Q. infectoria,* and *C. monogyna* (Bars indicate means ± SE; uppercase letters are indicated mean difference between in sampling dates; lowercase letters are indicated mean the difference between species, ANOVA followed by a Duncan's post-hoc test, $P < 0.05$)

Cambios estacionales en el potencial hídrico de mediodía (Ψmd) en *P. nigra*, *P. brutia*, *Q. infectoria* y *C. monogyna*.

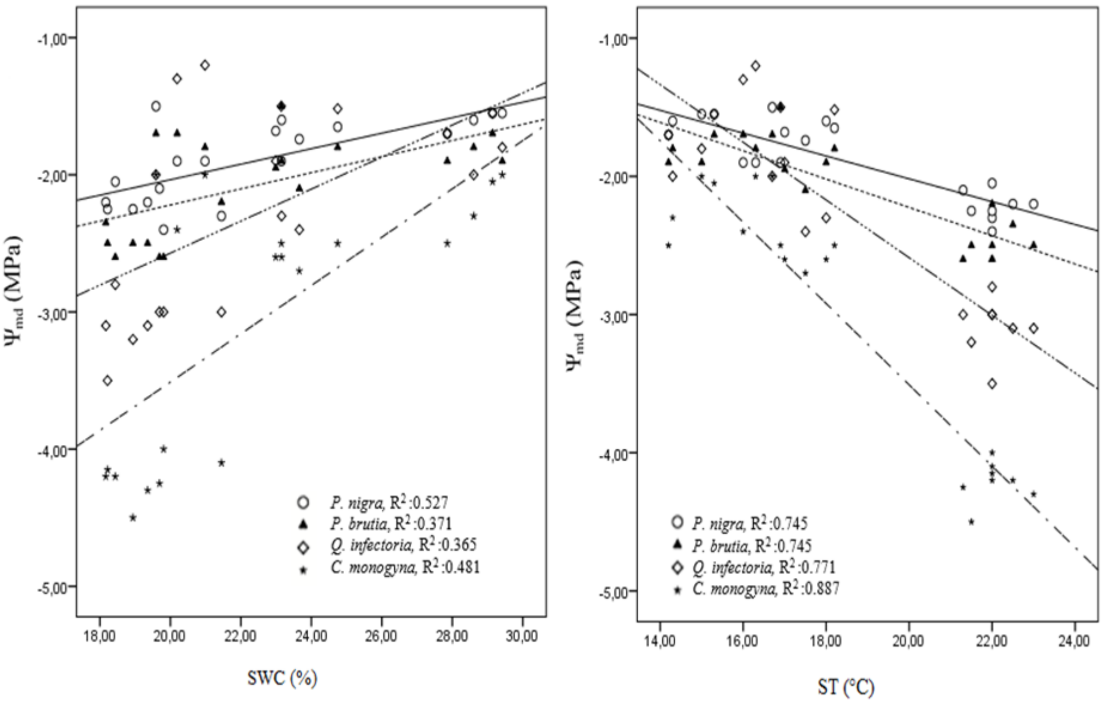

Figure 5. Relationships between midday water potential (Ψ_{md}), soil water content (SWC) and soil temperature.

Relación entre el potencial hídrico de mediodía (Ψmd), el contenido de agua del suelo (SWC) y la temperatura del suelo.

ted the highest amount of sugar. *Pinus brutia* accumulated less sugar than that accumulated by *P. nigra* in September. Sampling date had no significant effect on total soluble sugar in *P. brutia*, *Q. infectoria* and *C. monogyna* (figure 6). However, sampling date had a significant effect on proline content (table 1; $P < 0.001$).

P. nigra had the highest proline content in in June. *P. brutia* had the highest proline content in September. *Q. infectoria* had the highest proline content in May. *C. monogyna* had the highest proline content in May, June and September. *Pinus nigra* had a reduction in proline content from June to July-August, while *P. brutia*, *Q. infectoria* and *C. monogyna* had a reduction in proline content from May to July-August. However, there was an increase in proline content in all species starting from September. *Pinus brutia* and *C. monogyna* had the lowest proline content in July and August, while *P. nigra* and *Q. infectoria* had the lowest proline content in August (figure 7). Midday water potential was positively correlated with proline content (respectively $R^2 = 0.402$, $R^2 = 0.375$, $R^2 = 0.664$; $P < 0.001$), which was negatively correlated with soil temperature (respectively $R^2 = 0.508$, $R^2 = 0.461$, $R^2 = 0.598$, $P < 0.001$) in *P. brutia*, *Q. infectoria* and *C. monogyna*. Proline content was correlated with soil water content in *Q. infectoria* ($R^2 = 0.530$, $P < 0.001$) and *C. monogyna* ($R^2 = 0.417$, $P < 0.001$). There was a significant difference in proline content at all sampling dates, except in July and August (table 1; $P < 0.05$). *Pinus nigra* had

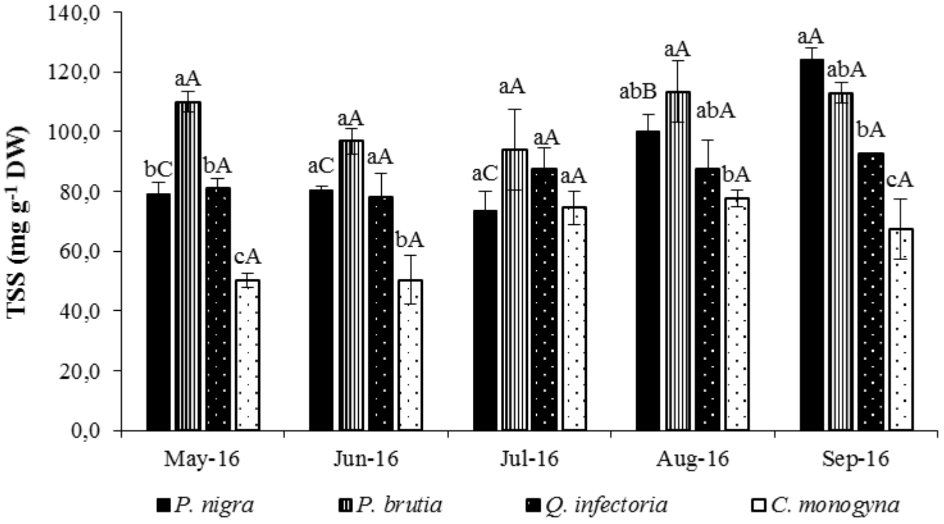

Figure 6. Seasonal changes in total soluble sugar content (TSS) in *P. nigra, P. brutia*, *Q. infectoria* and *C. monogyna* (Bars indicate means ± SE; uppercase letters are indicated mean difference between in sampling dates; lowercase letters are indicated mean the difference between species, ANOVA followed by a Duncan's post-hoc test, $P < 0.05$).

Cambios estacionales en el contenido de azúcares solubles totales (SST) en *P. nigra*, *P. brutia*, *Q. infectoria* y *C. monogyna*.

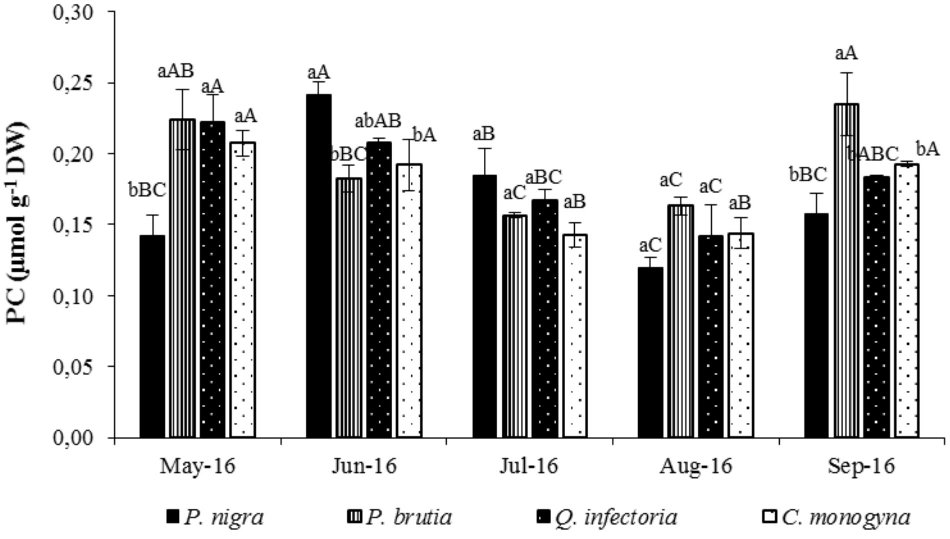

Figure 7. Seasonal changes in proline content (PC) in *P. nigra, P. brutia*, *Q. infectoria* and *C. monogyna* (Bars indicate means ± SE; uppercase letters are indicated mean difference between in sampling dates; lowercase letters are indicated mean the difference between species, ANOVA followed by a Duncan's post-hoc test, $P < 0.05$).

Cambios estacionales en el contenido de prolina (PC) en *P. nigra*, *P. brutia*, *Q. infectoria* y *C. monogyna*.

the lowest proline content, whereas the other species had similar proline content in May. The highest proline recorded in June was in *P. nigra*. *Pinus brutia* had the highest proline while the other species had similar proline content in September (figure 7).

Sampling date significantly affected chlorophyll a, chlorophyll b, chlorophyll a+b and carotenoid content in all species (table 1; $P < 0.001$). Chlorophyll a increased from May to June, slightly dropping in July and afterwards increasing again. Chlorophyll a was stable in *P. nigra* and *Q. infectoria* in August and September but decreased again in *P. brutia* and *C. monogyna* in September (figure 8A). Similar seasonal changes were observed in chlorophyll b, chlorophyll a+b and carotenoid (figure 8B, 8C, 8D). There was a significant difference in chlorophyll a, chlorophyll b, chlorophyll a+b and carotenoid content at all sampling dates (Table 1; $P < 0.001$). *Pinus nigra* and *P. brutia* had similar chlorophyll a, chlorophyll a+b and carotenoid content at all sampling dates except in August, when they were lower than those of *Q. infectoria* and *C. monogy-*

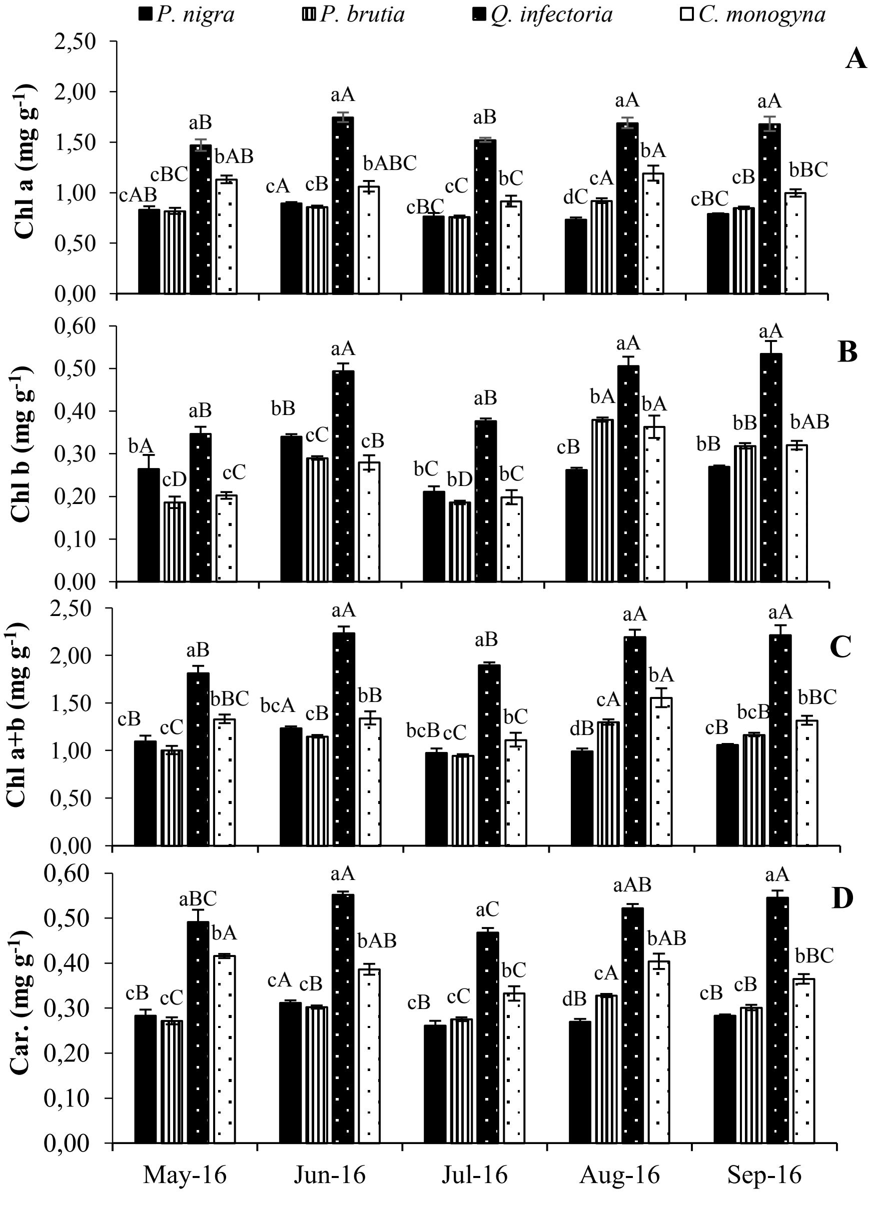

Figure 8. Seasonal changes in A) chlorophyll a (Chl_a), B) chlorophyll b (Chl_b), C) total chlorophyll (Chl_{a+b}), D) carotenoids (Car.) in *P. nigra, P. brutia*, *Q. infectoria* and *C. monogyna* (Bars indicate means ± SE; uppercase letters are indicated mean difference between in sampling dates; lowercase letters are indicated mean the difference between species, ANOVA followed by a Duncan's post-hoc test, $P < 0.05$).

Cambios estacionales en A) clorofila a (Chla), B) clorofila b (Chlb), C) clorofila total (Chla+b), D) carotenoides (Car.) en *P. nigra*, *P. brutia*, *Q. infectoria* y *C. monogyna*.

na. In August, *Q. infectoria* had the highest chlorophyll a, chlorophyll a+b and carotenoid content followed by *C. monogyna*, *P. brutia* and *P. nigra.* However, *C. monogyna*, *P. nigr,* and *P. brutia* had similar chlorophyll b at some sampling dates. *Quercus infectoria* had the highest chlorophyll a, chlorophyll b, chlorophyll a+b and carotenoid content at all sampling dates (figures 8A, 8B, 8C, 8D).

DISCUSSION

This study had three significant results: (1) There was a difference in responses of physiological and biochemical parameters to summer drought stress between coniferous and broad-leaved species. (2) *P. nigra* and *P. brutia* were more sensitive to summer drought than *C. monogyna* and *Q. infectoria* under the same environmental conditions. (3) The species had similar physiological parameters however different biochemical parameters. Midday water potential was high in *P. nigra, P. brutia, Q. infectoria* and *C. monogyna* in May, which is a rainy month. There was reduction in soil water content and increase in soil temperature with increase in temperature and decrease in precipitation. However, midday water potential decreased in the four woody species from May to July. The broad-leaved species had considerably lower Ψ_{md} than the coniferous species during the transition from the rainy to the dry season. *P. nigra* and *P. brutia* showed milder decreases in their midday water potential; while *C. monogyna* showed a larger decrease in its midday water potential during the dry season. The reduction in midday water potential may be related to the stress from low soil water content and high soil temperature (Ψ_{md} and SWC are significantly related). Plants keep the water potential as high as possible to avoid drought or tolerate low water potential to maintain drought conditions (Chaves *et al.* 2003). The differences in midday water potential between the species may be due to differences in stomatal regulation patterns (morphological, physiological, etc.). We can state that *P.nigra* has a higher midday water potential than that presented by *P. brutia* in the dry period (August). This may be because *P. nigra* closes its stomata earlier to prevent the leaf water potential from decreasing under drought. We can also state that the species increase their total soluble sugar content during the dry period and at the end of it. Overall, *P. nigra* and *P. brutia* accumulated more total soluble sugar than that accumulated by *Q. infectoria* and *C. monogyna* during this period. In other words, *P. nigra* and *P. brutia*, which have a higher midday water potential in the dry period, have a higher total soluble sugar content than that of *Q. infectoria* and *C. monogyna*. This may be about the ability of soluble sugars to maintain leaf water status and the osmotic adjustment of species under drought stress. Deligöz and Cankara (2020) reported that *P. brutia* had higher total soluble sugar than that found in *P. nigra*, which is similar to our result. Plants under water stress increase total soluble sugar (Holland *et al.* 2016). There were seasonal variations in total soluble sugar content in the four woody species. The change in total soluble sugar may be due to differences in seasonal growth exhibited by different species. Proline content is another response to drought stress and accompanied by a decrease in water potential of plant tissues (Irigoyen *et al.* 1992).

Proline content increased in all species at the end of the dry period (September). At all sampling dates, the highest proline content differed from species to species. This may be related to metabolic changes by the species under stress. *Q. infectoria* and *C. monogyna* had low proline content at the end of the dry period, which is probably because the species did not experience enough stress to accumulate soluble sugar and proline. The species tolerated low water potential to sustain short-term drought conditions. Näsholm and Ericsson (1990) reported that *P. sylvestris* had high proline content in early spring and low proline content in summer, spring and autumn, although proline content in *M. macclurei* and *S. superba* leaves was not affected by short-term drought (Kuang *et al.* 2017). Proline accumulation is positively correlated with drought tolerance (Van Heerden and De Villiers 1996). Chlorophyll differs among different species, functional groups and communities. *Q. infectoria* had the highest chlorophyll a, chlorophyll b, chlorophyll a+b and carotenoid content while *P. nigra* and *P. brutia* had the lowest chlorophyll a and chlorophyll a+b content compared to the species. High chlorophyll content under dry conditions indicates the severity of stress on plants and reduced leaf area (Yavaş *et al.* 2016). The oak leaves were more drought-resistant than the pine needles. Severe stress resulted in reduced carotenoid concentrations in the pine needles (Schwanz and Polle 2001). And also broadleaved trees possessed higher chlorophyll content than that possessed by coniferous trees (Li *et al*. 2018). In general, the chlorophyll content of the species decreased during the dry period. Daily maximum temperatures in July are above 30°C. High temperature can disrupt chloroplasts structurally and functionally, resulting in a temporary or permanent reduction in chlorophyll accumulation (Cui *et al.* 2006). These results confirm that plants adjust their chlorophyll content to adapt to the environment.

CONCLUSIONS

Our results show that the four woody species adapt to the environment in the Mediterranean climate regions against short-term drought; nevertheless, they develop different adaptation strategies. Based on the total soluble sugar and proline content in the dry period, we can state that the species were probably subjected to osmotic adjustment during the short-term drought. *P. nigra* and *P. brutia* exhibited high midday water potential and total soluble sugar accumulation during the short drought period, while *Q. infectoria* and *C. monogyna* may have tolerated the low water potential and not experienced enough stress to accumulate total soluble sugar and proline. We can argue that *P. nigra* and *P. brutia* (evergreen coniferous) are more drought-sensitive than *C. monogyna* and *Q. infectoria* (deciduous broad-leaved). In other words, *C. monogyna* and *Q. infectoria* are better at coping with short-term drought than *P. nigra* and *P. brutia*. This study provides insights into how species respond to natural drought. We believe that these results will help to develop afforestation projects for trees threatened by drought due to global climate change and will lay the groundwork for assessment tools necessary to avoid long-term field trials.

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