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Investigation of physiological changes in the affected *Quercus brantii* stand by oak charcoal disease

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Abstract: The purpose of this work was to monitor the effects of the environmental factors include temperature, precipitation and sun radiation on some physiological aspects of *Q. brantii* trees in the forest stand involved with the oak charcoal disease during a growing season. We designed a systematic random sampling and all trees were examined for disease status. Our result showed that 70.96% of the trees with different intensities are affected by the charcoal disease. Accordingly, values of predawn leaf water potential (PWP), midday leaf water potential (MWP) and chlorophyll variables showed significant difference in classes of oak charcoal disease. Linear regression analysis showed that the values of PWP, MWP and chlorophyll are changing at the high temperature. Mean of canker length are 20.5 cm and 51.7 cm in class 2 and 3 respectively and there is a great relation between PWP value and canker length (r^2 = 0.914). Canker length also has a linear relation with MWP values (r^2 = 0.627). Drought stress affected the physiological functions of oak trees and considerably reduced their defense potential against pathogen agents.

Keywords: leaf water potential; oak charcoal disease; linear regression; *Biscogniauxia mediterranea*; Zagros forests

The climate change scenarios for the 21st century have predicted changes in the ecosystems which will cause many difficulties for oaks to adapt to and mitigate these new environmental conditions (Borja et al. 2008; Lindner et al. 2010; Corcobado et al. 2014).

Endophytic fungi are sensitive to changes in temperature, humidity and host physiology. These changes make endophytic fungi change their population size, reproduction and distribution and these fungi are known as a live indicator of climate change (Boyer 1995).

The genus *Biscogniauxia* causing the charcoal disease in the Mediterranean and semi-Mediterranean oak trees has become one of the main problems of oak forests in the world (United States, Africa, Italy, Spain, Portugal, Turkey and Iran), especially in areas where climate change has occurred (Ju et al. 1998; Jurc, Ogris 2006; Mirabolfathy 2012). *B. mediterranea* can easily spread through large cavity vessels, colonise bark and woody tissues, and is able to kill the host in a single growing season (Mazaglia et al. 2001). *B. mediterranea* causes necrosis on stems and branches of *Quercus castaneifolia*, *Q. brantii*, *Zelkova carpinifolia* (Mirabolphathi 2013) and *Amygdalus scoparia* (Rostamian et al. 2016) in Iran.

Low water supply concurrent with high sunlight and high temperatures leads to severe drought conditions and outbreak of some diseases in these ar-

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eas (Bussotti et al. 2014). Plants are continuously affected by below- and aboveground abiotic and biotic stressors (Milanović et al. 2015).

Environmental stresses such as low or high temperatures and drought may lead to physiological plant modifications and influence the plant susceptibility to fungal pathogens (Schoeneweiss 1975; Boyer 1995; Garrett et al. 2006). Studies have been conducted in relation to the impact of environmental stress on physiological functions of trees. The effects of the causal agents of charcoal disease on the growth and physiological response of two-year-old seedlings of *Quercus brantii* were evaluated under drought stress in a greenhouse over a period of nine months. The survival was 21.7% lower in seedlings inoculated with *B. mediterranea* subjected to drought stress compared with the control treatment (Ghanbary et al. 2017). Endophytic behaviour of *Biscogniauxia mediterranea*, the causal agent of charcoal disease of oak, was studied on *Quercus cerris* in a forest in central Italy over two growing seasons. PWP and MWP values in July to August 2003 were significantly lower than those in 2002 (unpaired *t*-test, $P < 0.0001$) (Vannini et al. 2009).

Many studies have been conducted on *B. mediterranea* to investigate its endophytic behaviour on several Mediterranean oak species and to determine its distribution, morphobiological (Anselmi et al. 2000; Linaldeddu et al. 2005), ecological (Turco et al. 2004) and biomolecular (Luchi et al. 2005) characteristics. In addition, numerous studies have been carried out on the effects of stresses such as drought and disease on oak seedlings (Luque et al. 1999; Linaldeddu et al. 2009; Arend et al. 2011; Zolfaghari et al. 2013; Ghanbary et al. 2017). However, the effects of environmental factors on the physiology of healthy and diseased trees in forest stand have not been sufficiently evaluated.

The purpose of this study was to monitor the effects of environmental factors including temperature, precipitation and solar radiation on some physiological aspects of *Q. brantii* trees in the forest stand affected by the oak charcoal disease during a growing season.

MATERIAL AND METHODS

The area under study. The study area is the Kakasharaf forest of Lorestan located in the Zagros Mountains, western Iran. The size of the study area is 430 ha, and it is situated between 33°20'54'' to 33°22'10'' northern altitude and 48°29'23'' to 49°30'9'' eastern longitude (Fig. 1). Its mean annual precipitation is 496.4 mm. The altitudes of the region range from 1,390 to 1,510 m a.s.l. The

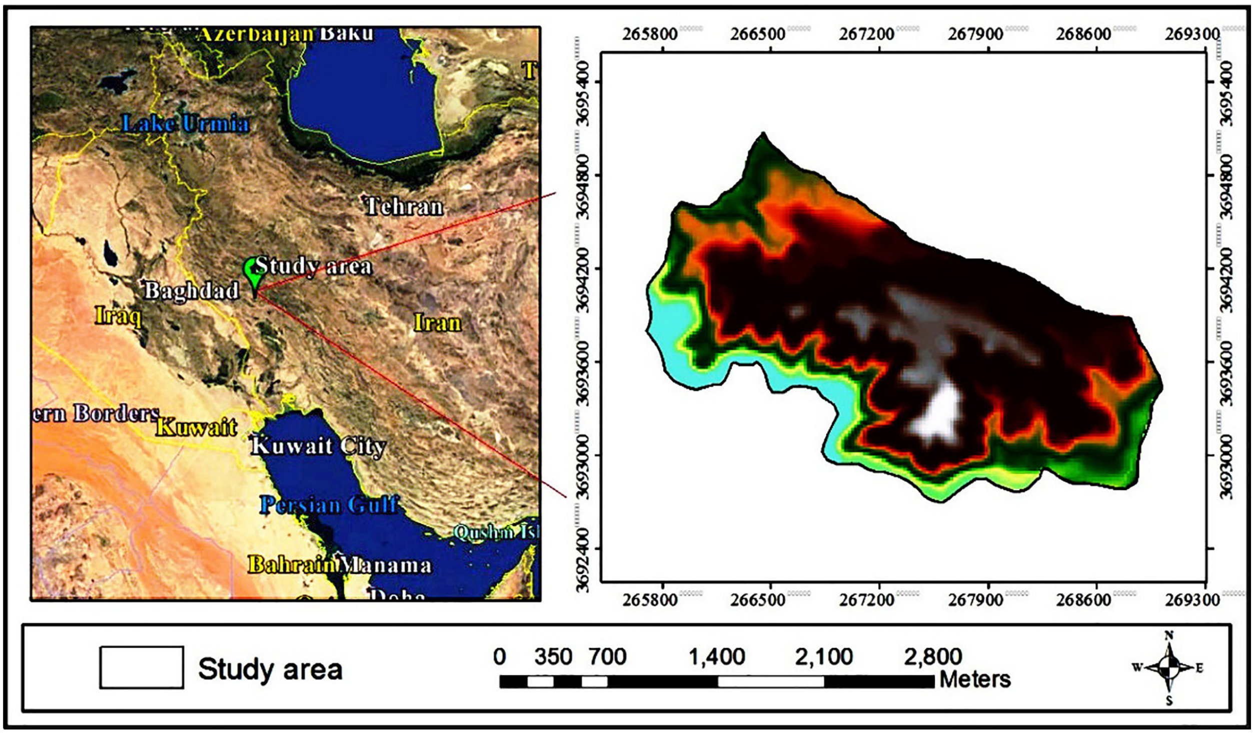

Fig.1. Study area of Kakasharaf Forest in Lorestan province

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main forest stand types in the study area are *Quercus brantii* on northern slopes and *Quercus brantii* with *Amygdalus scoparia* on southern slopes. Forest stand in the study area is infected by the charcoal disease. It is known as the focus of the *Biscogniauxia mediterranea* disease in Zagros forests.

Research methods. We designed a systematic random sampling with dimensions of 200 × 150 m in ArcMap 10.4.1 (ESRI, Redlands, USA). Then to inventory, circular plots of 1,500 m^2 in size were placed at the intersection of the sides of the grid. In each plot, all trees were examined for the disease status.

During the growing season 2016, we collected data from the Kakasharaf site. Predawn leaf water potential (PWP) and midday leaf water potential (MWP) of trees were measured once a month during the growing season (April to November), using a Scholander-Hammel pressure chamber (PMS instruments, Oregon, USA). Chlorophyll variables were also measured once a month using a SPAD 502 chlorophyll meter (Konica Minolta, Osaka, Japan).

Averages of 10 leaf samples from different parts of the tree crowns as PWP, MWP and chlorophyll values were recorded.

The tree status in terms of the charcoal disease was divided into three categories: (*i*) asymptomatic – class 1, (*ii*) only limited exudates and symptoms of the fungal activity of *B. mediterranea* (discoloration and browning of leaves, drying of foliage, viscous liquid exudates observed on trunks) were found – class 2, (*iii*) exudates and symptoms of the fungal activity plus beetles and deep canker (caused by the beetles and an associated fungal pathogen) and wood-eating beetle activity have increased the range (McPherson et al. 2005; Kelly et al. 2008; Mirabolfathy et al. 2012) – class 3.

The nearest synoptic station to the study area was Khorramabad synoptic station and averages of temperature (Max, Min and Mean), rain and sunshine hours every month as weather variables were obtained from this station.

Diagnosis of diseased trees. Morphological and molecular methods were used to detect *B. mediterranea* in trees. By comparing the characteristics of anamorphic isolates with the characteristics mentioned in the identification keys (Ju, Rogers 1996; Ju et al. 1998; Ju, Rogers 2001; Mirabolfathi 2012) the causal agent of the charcoal disease was identified. The molecular method was also used to confirm the species identification. The identity of *B. mediterranea* isolates was confirmed by PCR amplification with specific primers. Primers for the DNA amplification of *B. mediterranea* were designed using Primer Express Software Version 3 (Applied Biosystems) (Rostamian et al. 2017).

Analysis methods. A multiple linear regression model was created to examine the relationship between chlorophyll and the difference between PWP and MWP with weather variables. Moreover, linear regression was carried out to study the relationship between canker length with PWP and MWP. Data sets were previously tested for normality by the Kolmogorov-Smirnov test. All statistical analyses were carried out by R 3.5 (R Core Team, Auckland, New Zealand).

RESULTS

The results showed that a total of 2,863 trees were inventoried, while 29.04% in class 1, 40.18% in class 2 and 30.78 in class 3 were found. Accordingly, 70.96% of the trees with different intensities were affected by the charcoal disease. The values of PWP, MWP and chlorophyll variables showed significant differences in disease classes (Fig. 2).

During the growing season from April to November, values of PWP in class 1 were always higher than those in the other two classes and the lowest PWP values were in class 3 (Fig. 3a). The highest PWP values were observed in April and November and the lowest in June to September. Similarly to the PWP values, MWP values were also highest in class 1 and the lowest MWP values were observed in class 3. In April and November MWP values

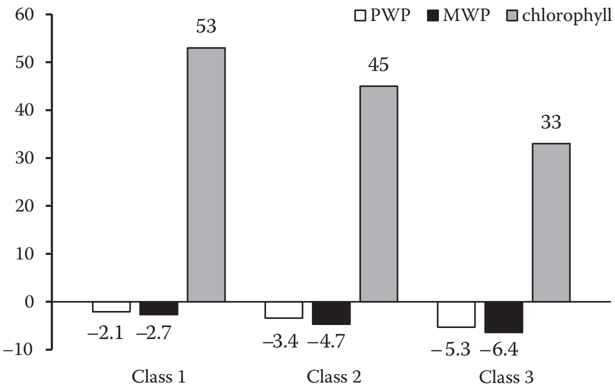

Fig. 2. Average values of PWP, MWP and chlorophyll variables in the charcoal disease classes

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were highest and in July to September they were lowest (Fig. 3b).

Chlorophyll values in class 1 and class 2 were almost similar, but chlorophyll values in class 3 were lower than those in the other two classes. Chlorophyll had an increasing trend since the beginning of the season, and peaked in July and August, then this trend decreased until it reached its lowest level in November (Fig. 3c).

The MWP values are always lower than the PWP ones. A difference between PWP and MWP is different in trees. This difference can be due to various factors, including physiological factors and environmental factors. Linear regression analysis shows the relationship between the PWP and MWP differences with stress factors in the area under study, including precipitation, temperature and sunshine hours in the charcoal disease classes (Table 1). In model 1, linear regression showed that Max and Min temperature variables were related to the PWP and MWP difference in class 1 of charcoal disease (r^2 = 0.693, P < 0.001). In this model, the Max and Min temperatures have a positive sign, so increasing their values will increase the difference between PWP and MWP. In model 2, a linear relationship was found between the PWP and MWP difference with precipitation and Max temperature in class 2 of charcoal disease (r^2 = 0.787, P < 0.05). The precipitation variable has a negative sign, so decreasing its value will increase the PWP and MWP difference. On the other hand, increasing the Max temp will increase the PWP and MWP difference. In class 3 of charcoal disease there was not observed any significant linear relationship between the PWP and MWP difference and precipitation, temperature and sunshine hours (P > 0.05).

In model 4 and in class 1 of charcoal disease, a significant relationship between chlorophyll and mean temperature was observed (r^2 = 0.881, P < 0.001). In this relationship, increasing the mean temperature in the area under study will increase the chlorophyll content. Model 2 showed that there was a relationship of the chlorophyll variable with sunshine hours and mean temperature (r^2 = 0.976, P < 0.001) in the

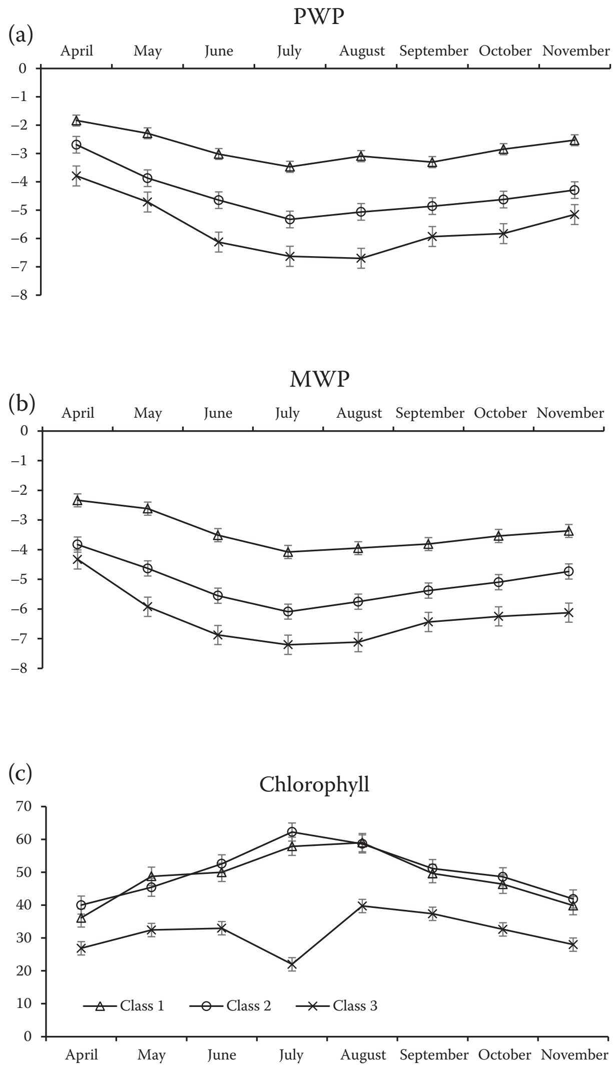

Fig. 3. Average values of PWP (a), MWP (b) and chlorophyll (c) variables in the charcoal disease classes during the growing season

Table 1. Results of linear regression for the relationship between PWP and MWP differences and chlorophyll with the stress factors in the area under study

Model	Dependent variable/disease class	R^2	Standard error	Sig.	Equation
1	PWP–MWP 1	0.693	0.120	0.049	= 2.797 – 0.074 Min Temp + 0.076 Max Temp
2	PWP–MWP 2	0.787	0.129	0.021	= –0.605 – 0.008 Rain + 0.03 Max Temp
3	PWP–MWP 3	0.318	0.28	0.15	= 0.671
4	Chlorophyll 1	0.881	2.929	0.001	= 20.405 + 0.88 Mean Temp
5	Chlorophyll 2	0.976	1.418	0.000	= 3.042 + 0.07 Sun – 1.816 Max Temp
6	Chlorophyll 3	0.758	3.33	0.029	= 153.535 – 8.776 Max Temp + 6.301 Mean Temp

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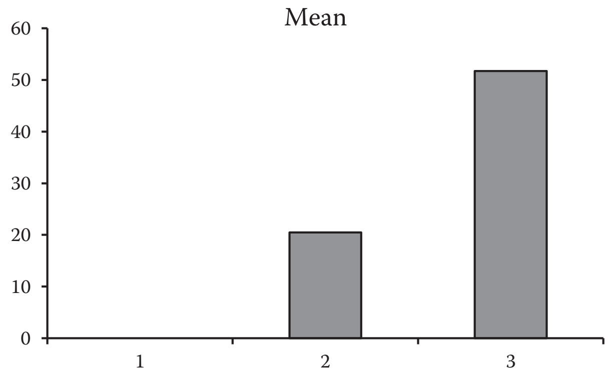

Fig. 4. Average of canker length in charcoal disease classes

trees of class 2 and model 3 showed a linear relation between the chlorophyll variable and maximum temperature and mean temperature (r^2 = 0.758, P < 0.05) in class 3 of charcoal disease.

There was a significant difference in the mean of canker length between charcoal disease classes. With the development of charcoal disease in trees, the length of the canker also increased. The mean of canker length was 20.5 cm and 51.7 cm in class 2 and 3, respectively (Fig. 4).

The results indicate that the variation of PWP is related to the length of the canker. There is a strong relationship between the PWP value and canker length (r^2 = 0.914), so that with an increase in the canker length, PWP values will decrease (Fig. 5a). The canker length also has a linear relation with MWP values and this relation is inverse (r^2 = 0.627) (Fig. 5b).

DISCUSSION

In the present study we observed that PWP, MWP and chlorophyll values were different in the classes of oak charcoal disease. In other words, diseased trees showed lower PWP, MWP and chlorophyll values than healthy trees and with further development of the disease in the trees the values of these variables can also be reduced. In the growing season the values of PWP, MWP and chlorophyll variables were influenced by environmental factors including temperature, precipitation and sunshine hours. With the arrival of the summer season and especially of the months of July to August, these changes of values were more visible by increased temperature and reduced precipitation (Vannini et al. 2009).

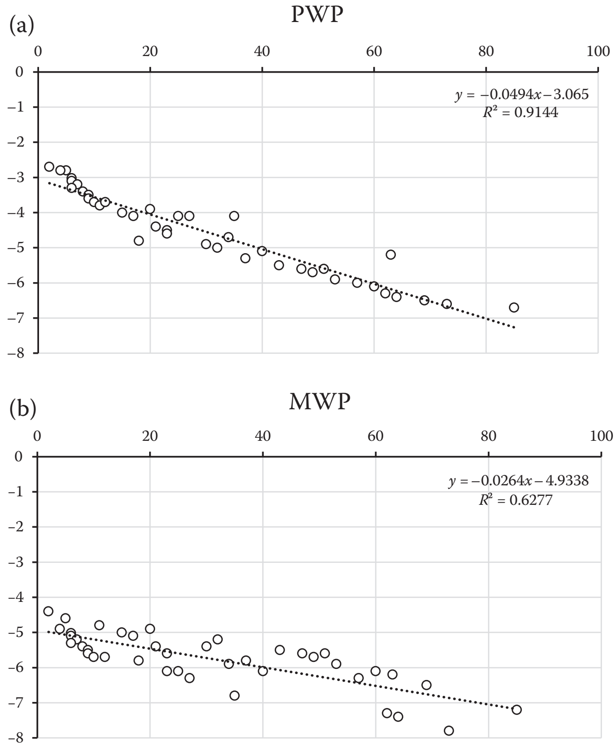

Fig. 5. Results of linear regression for the relationship between PWP (a) and MWP (b) with canker length

Changes in the water leaf potential from morning to noon were reduced due to temperature and precipitation conditions and this reduction was obvious in all healthy and diseased trees and according to the results a maximum of temperature is the most important factor associated with these changes (Schoeneweiss 1975; Boyer 1995; Garrett et al. 2006). But in class 3 of oak charcoal disease it was observed that the environmental factors including temperature, precipitation and sunshine hours did not show a regression equation by reducing the potential difference between PWP and MWP. It seems to be the reason why the development of the disease in trees has a greater impact on a potential difference between PWP and MWP than do environmental conditions. This can be seen in relation to canker length and PWP and MWP. The canker length has a strong relation with PWP values. While in the morning and measuring the PWP there is not a temperature change and the canker length that indicates the development of the disease in trees is the most important factor associated with PWP. In the midday the temperature is maximum, these temperature changes cause a reduction of the leaf water potential. According

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to the regression results the development of canker in host trees is more effective on the difference in leaf water potential than temperature changes. In model 3 of regression relations (Table 1), it is observed that the water potential difference does not show any relationship with environmental factors. It may be due to the fact that in the trees with a high level of charcoal disease, canker lengths are more effective than environmental factors (Ghanbary et al. 2017).

The charcoal disease pathogen can easily spread through large cavity vessels, colonise bark and woody tissues, and then cause the occurrence of the canker and its development in the trees. Some pathogens spread in the host vascular system through functional vessels, without their embolization. For example, *Ophiostoma ulmi* is able to take advantage of the transpiration stream to carry its propagules and diffuse longitudinally (Campana 1978). However, *B. mediterranea* appears to spread mainly by mycelial growth in the vessels (Vannini 1990) when the host is water stressed. Mycelial growth in the vessels seems unlikely to occur without "air seeding" (Zimmermann 1983) and, hence, the formation of emboli in the vessels. However, it is possible that, after penetration of the vascular system, *B. mediterranea* increases the extent of xylem embolism, in which case the decline of infected trees may be caused, wholly or in part, by a lack of xylem functionality and, hence, transport of water to the crown (Vannini, Valentini 1994).

Global or local climatic changes that induce stress in plants and a loss of adaptation to a particular environment could result in the interaction between a fungus and its host changing from saprophytism to parasitism. The close relation between environmental stresses and susceptibility of *Q. brantii* to *B. mediterranea* provides a useful model that is probably applicable to other host-pathogen interactions, where the pathogen is a weakness parasite.

CONCLUSIONS

In recent years, the climate change and decreased precipitation occurred in the western region of Iran, resulting in drought stress, followed by a widespread decline and death of oak forests. Drought stress affected the physiological functions of oak trees and considerably reduced their defence potential against pathogenic agents. Increasing temperature and decreasing precipitation will contribute to the spread of charcoal disease. The charcoal disease agent causes the physiological changes and development of canker length in the forests.

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