DESERT

Desert
Online at http://desert.ut.ac.ir

Desert 24-1 (2019) 43-49

The effect of simulated dust storm on wood development and leaf stomata in *Quercus brantii* L.

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Received: 11 June 2018; Received in revised form: 31 December 2018; Accepted: 20 February 2019

Abstract

Dust storms are increasingly threatening the forest ecosystem in Western Iran. Due to its coverage of vast area of Zagros forest, the *Quercus brantii* is at the front line of the attack. Most tree deaths in this forest are from this species. This study aims to investigate the effect of simulated dust on wood development and leaf stomata from seedlings of Persian oak. The oak seedlings were put in a chamber and dusted from 9 a.m. until 6 p.m. at three different days with an interval of 12 days. They were under three increasing concentrations of dust (5000, 7000, and 9000 mg/m^3). Leaf and wood samples of about 10 seedlings were collected, and the leaf stomatal features, growth ring and vessel features of both treated and untreated seedlings were examined. The results showed no variations in stomatal features except for pore aperture. As for wood features, growth ring, vessel number and diameter considerably changed. In treated seedlings, the growth ring and vessel diameter were smaller and the number of vessels was higher than in the untreated seedlings. No detection of differences in stomatal features could be tracked due to the short-period of treatment. The usual seedlings' annual growth could be altered by treatment affecting on both annual growth ring and vessel features. To sum up, it can be concluded that dust storm has strong effect on seedling's growth as can limit wood formation.

Keywords: Dust Storm; Wood Anatomy; Leaf Features; Growth Ring; Persian Oak

1. **Introduction**

Dust phenomenon has been considered as a serious natural threat during the past few decades (Omidvar, 2006). In fact, dry and semi-dry lands in the world have been expanding due to the increasing process of global warming global increasing in Earth's temperature, reduction in precipitation, climate change, increased evapotranspiration, as well as excess and misusage of water resources in different activities carried out by mankind. These conditions give rise to new sources of dust and amplify the influence of dust storms (Liu *et al.*, 2003). Tiny dust particles in the air - “particulate matter" (PM) - is a kind of air pollution that comprises of a mixture of some varying particles suspending in the atmosphere. In general, PM is a complex of fine solid materials like soil dust, different types of micro-organisms, ashes, plant pollens, soot, and many other minute particles. PM may arise from different sources such as anthropogenic activities or natural sources (Schelle-Kreis *et al.*, 2007). In the Middle East, especially in Iran, most dust storms originating from the deserts of neighboring countries like Saudi Arabia, Syria, and Iraq destructively attack the West and South-West of the country and have had enormous effects on many aspects of plant, animal, and human life (Omidvar, 2006). In addition, about one-fourth of Iran is located in the dry and desert belt of the world, therefore they are inevitably attacked by dust storms. However, the severity and duration of the local storms are too weak and are not as important as the storms originating from neighboring countries (Ghaffari and Mostafazadeh, 2015).

Plant vigor and performance are affected by environmental stresses. Many researches have particularly studied the morpho-physiological responses of plants and their organs function against dust pollutions (Chaturvedi *et al.*, 2012;

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Singh and Rao, 1981), but there are few studies that show changes in anatomical features. Insights into plant variations against environmental changing have mainly been recognized through cellular comparative studies between affected and non-affected plant individuals on contrasting sites or situations. Yet, a plant's growing response to environmental conditions is a key factor in determining whether that species can persist environmental changes or is influenced detrimentally by them (Ben Rejeb *et al.*, 2014).

Understanding the anatomical features of a leaf helps better identify the effect of pollutants. The absorption of particulate matters is related to leaf structure. The density and size of the stomata indicate the leaf adaptation to pollutants (Gostin, 2009). The size and frequency of trichomes is of key trait to take and keep the dust (Muneer *et al.*, 2013). Stomatal conductance is influenced by environmental factors, position at the canopy, and the age of the leaves. Dust-covered leaves receive less light for photosynthesis, which in turn affects leaf stomatal conductance, plant biomass, and the rate of photosynthesis. Furthermore, leaves covered with dust absorb more solar radiation that increases leaf temperature (Wijayratne *et al.*, 2009). In addition, fine dust may clog the stomatal openings leading to adverse effect on photosynthesis and leaf temperature. Other factors such as sandblasting result in the loss of plant leaves which decrease photosynthetic activities and production of grains or fruits (Zia-Khan *et al.*, 2015). Shrivastava and Mishra (2018) reported changes in the stomatal size of some plants induced by air pollution.

Persian oak (*Quercus brantii*) trees are one of the most important woody plant species in the Zagros forest, in Western Iran (Browicz, 1982). This tree acts as a great filter for purifying air from pollutants, particularly dust storm. For thousands of years, they have adapted to habitats, thus small changes in environmental conditions would affect their performances. Unfortunately, great numbers of trees have disappeared in wide parts of these forests because of destructive parameters (Moradi *et al.*, 2017). Our observation based on field trips has shown a high rate of Persian oak mortality in different sites.

To the best of our knowledge, there is no specific report on the effect of dust storms on leaf stomata and wood anatomical properties. Therefore, due to lack of laboratory information, we selected Persian oak (*Quercus brantii* Lindl.) to examine leaf stomata and wood radial growth variation to simulated dust storms.

2. Materials and Methods

A total of 30 two-years-old Persian oak (*Q. brantii*) seedlings were provided from Eyvan Nursery, a main governmental seedling provider in Ilam, Iran. The seedlings were then transported to the pilot nursery of Ilam University (Table 1). The plastic pots were replaced by bigger holding of about 3 kg air-dried soil with a mixture of plow layer of field soil: manure: gravel (2:1:1). To adapt with the new situation, appearance, and maturation of the leaves, the seedlings were placed in the yard and irrigated by tap water for three months.

Table. 1 The basic information of study place in University of Ilam, Iran

Latitude	Longitude	Altitude (m) a.s.l	Ave. Pre. mm	Min. T. °C	Max. T. °C
33° 21′ 30″	45° 41′ 07″	1427	581	-13	42

Ave. Pre. = Average Precipitation; Min. T.= Minimum Temperature; Max. T.= Maximum Temperature

2.1. Dust preparation

The dust for the experiment was collected from the desert around the city of Dehloran, southern part of Ilam. The desert is very close to that of the neighboring country's (Iraq) that likely is more similar to the dust originated from natural sources. In the laboratory, the dust was ground and passed through a series of sieves meshes numbers of 35, 80, 200, and 400. Finally, dust particles with the size of less than 40 µm were collected. The size of fine dust affecting the plants' organs is usually less than 40 µm (Shams and MohamadZadeh, 2013). Some characteristics of prepared dust are presented in the Table 2 that are almost similar to the dust with Arabian origin (Modaihsh, 1997; Modaihsha and Mahjoub, 2013).

Table 2. Some chemical characteristics of prepared dust

Sand %	Silt %	pH	EC (ds/m)	$CaCO_3$ (%)	$CaCO_4$ (%)	Cl^- Mg/L	Texture
49.16	39.18	7.36	2.53	51.75	30.10	0.8	Loam

EC= Electrolyte Conductivity

2.2. Simulated Dust Chamber

In order to investigate the effect of dust on the Persian oak seedlings, a chamber with the dimensions of 200×200×200 cm was designed. After leaf maturation on the last days of May, the seedlings were put inside the dusting chamber. For longer suspension of dust, we provided three small barbeque fans at different parts of the chamber. In addition, to avoid any stress on the seedlings, the air flow did not contact the leaves. The chamber was covered by polyethylene plastic sheet and to ventilate the room, some small holes were made at two corners of the chamber. To prevent the adverse effect of the sun on the seedlings, the chamber was put under a covered yard. Similarly, a same chamber was provided for the untreated (control) seedlings.

2.3. Dusting Process

The dusting process was taken out for three periods. At first, the seedlings with a diameter of about 1 cm and a height of about 100 cm were treated during one day by about 220 g dust from 9 a.m. until 6 p.m. for six series of 1.5 hours. During each series of dusting, about 36 g of dust was added to the chamber to maintain the dust concentration. Likewise, 10 seedlings as control group were put inside the control chamber. The second and third periods of dusting were also similar to the first one, but with an interval of 12 days between the periods and with an increased dust concentration of 330 and 440 g. According to the Department of Environment, Ilam (2017), the concentrations of dust in the air of Ilam in most of the polluted days (more than 50 days from April-November) have been about 5000 mg/m^3 and more. The dust concentrations for three dusting periods were 5000, 7000, and 9000 mg/m^3, respectively. One week after the last period, some wood specimens were taken from the stem, five cm above the collar of treated and untreated seedling samples. Meanwhile, for stomata analyses, some matured leaves from middle part of the crown were sampled from both treated and untreated seedlings.

2.4. Wood Samples Processing and Variables Measures

To obtain more detailed information on the effects of dust storms on the wood structure of Persian oak, the stems of 10 seedlings were cut into blocks with about 10×5×5 mm, and then a microscopic slide was prepared from each seedling as following: To soften the wood samples, they were first boiled for about 30 minutes and then 20-25µm thick transverse sections were cut by a rotary microtome (MK 1110, China). The sections were stained by Safranin O and dehydrated by a series of ethanol from 50% up to 100%. The stained sections were transferred onto the glass slide and mounted with Canada balsam glue and analyzed under a light microscope (Olympus, CX 22), equipped with a digital microscope camera. Image acquisition and measurements were performed by the software (TrueChrome Metrics). Only the last growth ring attached to the bark was considered. Measures were averaged for the following variables: width of growth ring; the number; the seriate; and the tangential diameter of early-wood vessels.

2.5. Leaf Sample Preparation

Due to lack of stomata on the adaxial surface of the leaf blade, the study was merely focused on the abaxial surface. Small pieces of the leaf were cut with a dimension of about 5×5 mm from the middle part and near the mid vein of the leaf blade. There was some difficulty to observe the stomata on the abaxial surface due to huge numbers of trichomes. Therefore, they were removed by a safety razor and paper adhesive tape. To remove the chlorophyll, the leaf samples were boiled in a mixture of acetic acid and hydrogen peroxide (1:5) for 60 min. The bleached samples were stained by Safranin O for 15 min. and then dehydrated by a series of ethanol of 85%, 95%, and 100%. Finally, permanent slides were prepared (Camargo and Marenco, 2011). The stomatal features, as Figure 1, were measured by light microscope with magnification of ×1000. The measurements of the stomata features were centered on 50 stomata.

2.6. Statistical analysis

The data were subjected to the Kolmogorov-Smirnov normality test. Paired sample t-test was used to determine any differences between tree ring features and stomatal parameters before and after treatments. The data were performed using SPSS, version 21 (for windows).

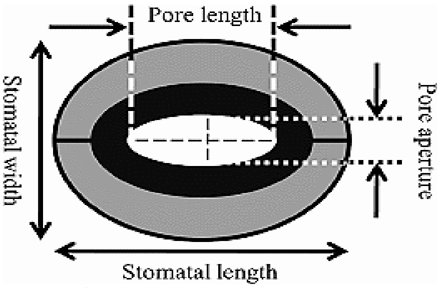

Fig. 1. Schematic representation of the stomatal and pore dimensions as measured by image analyzer

3. Results

3.1. Tree Ring Growth

In both, treated and untreated seedlings, growth rings formed during the progress of treatment. Whereas, a clear reduction in the width of annual ring of dusted seedlings was obviously observed (Figure 2 a-c). The average ring width of treated and untreated seedlings was 528 (±34) µm and 880 (±56) µm, respectively. Therefore, the growth ring in treated seedlings decreased by about 40% compared to the untreated ones.

3.2. Vessel Anatomy

The number of vessels within the annual ring increased in treated seedlings, from 15 (±2.7) in untreated to over 20 (±4.1) in treated seedlings. Furthermore, the tangential diameters of the vessels pore along the boundary of the rings were 252 (±27.2) µm to 206 (±18.7) µm in untreated and treated seedlings, respectively. The reduction of vessel pores was about 18%. Therefore, the high level of dust was associated with significant change in vessel features, as the number and diameter of vessels in untreated seedlings were, respectively, lower and bigger.

The ring porous nature of the *Quercus brantii* wood for generating vessels in some seriates close to boundary of the ring was clear both in treated and untreated seedlings. The treatment did not change the number of vessel seriate, as they were almost in 2-3 seriates (Figure 2 a-b).

3.3. Stomatal Variation

The Abaxial side of Persian oak is fully covered by stomata (Figure 3). Variations in stomatal parameters, including stomatal density (SN), stomatal length (SL), stomatal width (SW), pore length (PL), and pore aperture (PA) of *Q. brantii* showed that their responses to dusting were not associated with increasing the dust concentration (Table 3). As shown in the Table, the variation among the stomatal features were insignificant. Pore aperture were closed in treated compared to open stomata in control plants.

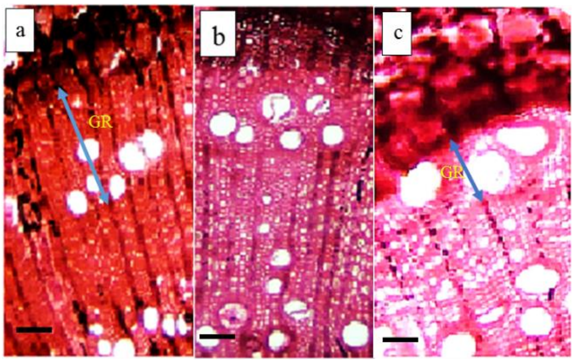

Fig. 2. Variation in wood of treated and untreated seedlings of *Quercus brantii* by dust storm in simulated condition. a: normal growth ring (GR) in untreated seedling. b and c: narrow growth ring in the treated seedlings. Scale bar=75µm

Table. 3 Mean value (±SD) of different features of stomata at seedlings treated and untreated by dust

Treatment	SN (N. µm^2)	SL (µm)	SW (µm)	PL (µm)	PA† (µm)
Treated	15.20 (±2.44)	21.02 (±2.85)	14.08 (±1.70)	10.60 (±2.10)	--
Control	14.62 (±1.98)	20.06 (±2.39)	15.32 (±1.93)	9.40 (±2.08)	1.06 (±0.42)

SN, Stomatal density; SL, Stomatal length; SW, stomatal width; PL, pore length; PA, pore aperture. Values are means of 50 replicates. SD, Standard deviation. Due to clogging of the pore by dust (Moradi *et al.,* 2017), it was impossible to measure their size by light microscope, therefore no value was recorded

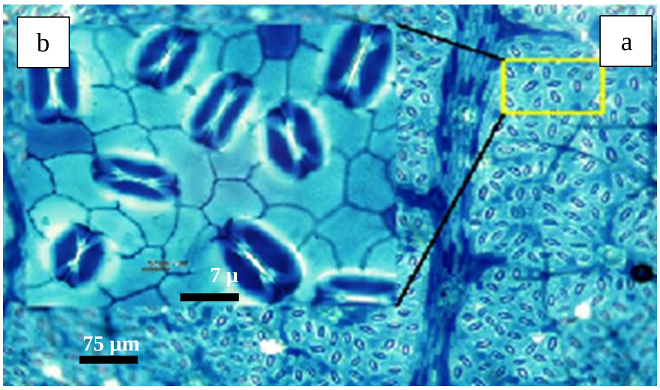

Fig. 3. The arrangement of stomata in untreated seedlings. a and b, low (×100) and high (×1000) magnifications of stomata

4. Discussion

The knowledge of a tree species' growth features and effects of environmental variables, as well as silviculture on it could be a key matter for evaluating and preserving the long-life of the forests. This is the case with the Persian oak (*Quercus brantii*) of Zagros forest which is under huge pressure of dust storms coming from domestic (dried lands of the country) and external (deserts of neighboring countries) resources that may be a cause of growth decline (Rasooli *et al.*, 2011). However, very little is known on Persian oak growth, mainly on the wood cells, their growth and development and nothing so far has been published on the response of wood anatomy to dust storms.

Generating the secondary tissues of the woody stem is an active process that is considerably influenced by whole-tree physiology, which is consecutively controlled by environmental parameters. The effect of these parameters on secondary growth is always recorded within the wood cells and revealed in the tree-ring structure (Scarpella and Meijer, 2004). In this study, an obvious variation of some wood biometric traits was observed in Persian oak seedlings influenced by dust deposition in the current year. Dust deposition on the oak leaves gradually changes the seedlings' activities, and such had a negative impact on ring width and vessel features. The formation of an appropriate network of vessels during generating annual wood ring is an essential parameter for effective water transport in trees. The reduction in the width of annual ring was pronounced in dusted seedlings. This suggests the weakening of water transportation to the leaves leading to lessen the growth of apical meristem as stated by Tulik *et al.*, (2017) on ash dieback. Furthermore, formation of no latewood from stress has also been reported by Asshoff *et al.*, (1999) in Spanish chestnut.

The results of this paper show that dusted seedlings produced vessels with reduced size but increased density. Environmental conditions occurring during the early wood vessel formation change the cell size by affecting the rate of cell division and differentiation (Fonti and García-González, 2004; Fonti and García-González, 2008). These adjustments via tree physiological alterations are to adapt to new conditions. Correspondingly, cambial activity and wood cell development are highly related to the accessibility of photo-assimilates. By inducing environmental stresses such as dust storms, the photosynthesis rate is decreased and assimilate translocation is adjusted, which finally impacts cambial activity (Eilmann *et al.*, 2006). Dust treatment can directly impact cells undergoing differentiation and consequently produce a lot more lignified cells around the vessel pores (Glerum and Farrar, 1966). In addition, the results of the current research are in line with the hypothesis that any decrease in growth ring and vessel size could be related to a change in hormonal regulation for cambial activity and wood formation (Aloni and Zimmermann, 1983). The large number of small vessels in the growth ring seems to be related to induced stress (Sousa *et al.*, 2015). There is an adverse relation between vessel number vs. vessel size and area as described by Pourtahmasi *et al.* (2011).

Accordingly, dust deposition should affect the availability of light for photosynthesis and clogs the stomatal pore for air exchange and thus increases stress on plant metabolism (Keller and Lamprecht, 1995; Anthony, 2001). In this case, Kushwaha *et al.*, (2018) stated that significant changes in the stomata size in plants affected by dust pollution. However, concerning the stomatal features, no changes were observed in the stomata from treated and untreated seedlings. This might be due to the short-time period of dusting, in which the seedlings had no enough time to adopt to the new condition induced on it. The only clear difference is related to the stomatal pore aperture as stated earlier in the results.

5. Conclusion

In this study, we investigated the effect of dust on current year growth ring, early-wood vessels and leaf stomata traits of Persian oak in a simulated condition of a dusting chamber. The results revealed that dust storm as an environmental pollution can adversely affect the oak tree growth with a reduction in the width of growth ring and vessel features. However, no changes in the leaf features were observed. To precise evaluation of alterations in leaf features, it may need to induce the dust storm in simulated conditions for longer period of time.

Acknowledgements

We would like to thank Miss Afrooz Havasi and Mr. Farshad Roushani Nia for their kind help and contribution for laboratory works, and also Prof. Nigel Chaffey from Bath Spa University for the valuable comments and suggestions on the manuscript. Furthermore, this paper benefitted from comments made by three anonymous reviewers.

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