Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 April 2021

Variations in Wood Density, Annual Ring Widths and Other Anatomical Properties of *Quercus brantii* Affected by Crown Dieback

Forough Soheili[1], Steve Woodward[2], Isaac Almasi[3], Hazandy Abdul-Hamid[4], Hamid R. Naji[1*]

[1] Department of Forest Sciences, Ilam University, Ilam, Iran.

[2] University of Aberdeen, School of Biological Sciences, Aberdeen AB24 3UU, Scotland, UK.

[3] Department of Statistics, Razi University, Kermanshah, Iran.

[4] Institute of Tropical Forestry and Forest Products (INTROP), Universiti Putra Malaysia, Malaysia.
[f] Faculty of Forestry and Environment, Universiti Putra Malaysia, Malaysia.

[*] Correspondence: Hamid Reza Naji, Department of Forest Sciences, Ilam University, Ilam, Iran. Email: h.naji@ilam.ac.ir

Abstract: Tree decline resulting from climate change results in physiological weaknesses, attack by harmful pests and pathogen and threatens forest ecosystem stability. In the work described here, the effects of drought on wood density, tree ring width and variations in vessel morphology was investigated in Persian oak (*Quercus brantii*) in the forests of the Zagros Mountains, Ilam province, western Iran. Radial discs were cut from trunks of declined and healthy trees and wood blocks cut radially from the sapwood, heartwood and juvenile core. Observations were made on transverse sections from the blocks using microscopy. In trees with decline symptoms, wood density was greater than the healthy trees. Furthermore, declining trees had narrowest ring width, reduced vessel diameter, vessel area, and highest number of vessels and tylose in pith towards bark. It was concluded that changes in anatomical features are associated with weakening of the trees and are components in declining tree health.

Keywords: Climate Change, tree decline, Wood density, Anatomy, *Quercus brantii*.

1. Introduction

Forest decline is a serious problem exacerbated by climate change, particularly in response to extended periods of drought [1]. Decline and mortality of oak species has been reported for more than one century [2], sometimes occurring in vast area of forests due to complex and interacting

© 2021 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 April 2021

reactions involving environmental stresses, pests and diseases [3]. The Zagros forest in western Iran is the largest oak forest in the world covering over 5 million ha in West of Iran, dominated by Persian oak (*Quercus brantii* L.). Forest decline has been reported in these oak forests, ultimately resulting in extensive tree mortality [4]. In the past decade, the impacts of climate change on the Zagros oak forests led to serious forest disturbance, including dust storms, fires, and pest and disease outbreaks, leading to decline in the main forest tree species of the region [5].

Knowledge of anatomy and function in secondary xylem is a key issue in understanding tree defenses against pathogens [6,7]. Tree ring width records, for example, can provide valuable information about the responses of trees to past environmental events [8, 9].

Regulation of vascular cambium activity over the years is crucial to the ability of trees to adjust to fluctuating environmental conditions and, therefore, is highly responsive to climate change. Annual ring structure, however, is surprisingly stable compared to other morphological characteristics of trees [10, 11]. Although variations in tree-ring structure are well understood, little is known of the intrinsic mechanisms responsible for the variations [12].

Water potential in xylem vascular is a key parameter that is widely used to monitor drought stress conditions throughout the plant [13]. The vessel diameters have an important role in spread of the pathogen within the plant. Meanwhile, the sap flow which has a fundamental role in the diffusion of certain pathogens within the plant, is strongly influenced by the dimensions of the wood vessels [14]. Anderegg et al. [15] and Giagli et al. [16] demonstrated decreased cambium activity, shortened periods of cambial activity, formation of narrower growth rings, and intra-annual wood density fluctuations resulting from abiotic stresses on the vascular cambium. Abiotic stresses such as dust storms, as consequences of climate change, are also known to affect annual ring widths and vessel diameters and widths in *Q. brantii* [17], possibly due to disruptions to hormonal regulation during wood formation [18].

In order to prepare evidence-based management plans for the protection of forests in the Zagros mountains, it is crucial to understand the importance of biotic and abiotic factors and the relationship between the two in forest decline. To the best of our knowledge, there has been no specific study examining this issue. The aim of the work reported here, therefore, was to improve understanding of the impacts of drought stress on the wood structure of *Q. brantii*, focusing here on annual growth increment and vessel features. Improving our understanding of how the environment influences the host and the pathogen *together*, will help predict performance of the forests during drought to provide practical solutions for forest management.

2. Material and Methods

2.1. Study areas

This study was conducted on dead and healthy Persian oak trees (*Quercus brantii* L.) affected by drought stress in three forest regions: Dareh shahr (A), Sheshdar (B) and Chavar (C) all in Ilam province, Western Iran (Figure 1). The trees were natural regeneration and were sampled from the sites with uniform conditions of habitat, altitude and topography, and were in the same biosocial classes. The dominant trees in the forests were *Q. brantii*, *Crataegus* sp., *Acer monspessulanum* and a shrub, *Daphne* sp. Important information from the study sites is given in Table 1.

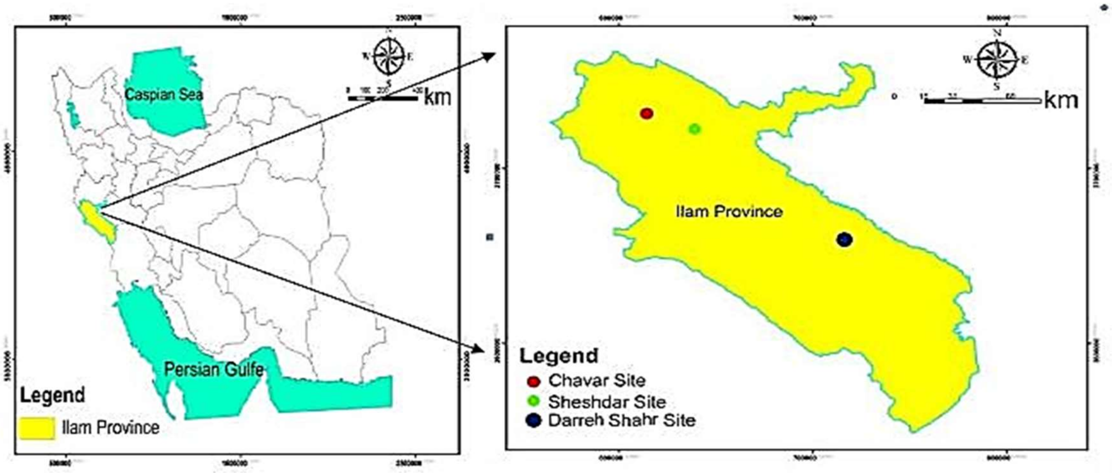

Figure 1. Location of the forests sampled in the Ilam province in western Iran.

Table 1. Basic characteristics of the *Quercus brantii* forests sampled for this work.

Forest Site	Altitude (m)	Longitude	Latitude	Ave. Precip. mm	Ave. Temp. ºC	RH %
Dareh shahr (A)	933	33° 3' 30" N	47° 19' 30" E	582.2	16.9	40
Sheshdar (B)	2230	33° 38' 55" N	46° 30' 37" E			
Chavar (C)	1680	33° 43' 03" N	46° 14' 36" E			

Ave. Precip.: Average precipitation; Ave. Temp.: Average temperature; RH: Relative humidity. Climate data were based on the synoptic meteorological station of Ilam city.

2.2. Sampling Method

The sampled trees in each site were located close to each other. As the forest is protected, no permission to cut trees in the stands could be obtained. Wood samples, therefore, were collected from the main trunks of trees felled by the Department of Natural Resources of Ilam Province under the plan to remove trees showing severe decline from the forest. Eighteen naturally regenerated trees (six trees from each site) of 70-90 years-old, with DBH ranging from 30-40 cm were selected. From each sample tree, a disc of approximately five cm in thickness was taken for further measurements. In the laboratory, each disc was carefully halved transversely, to give two discs for examination of wood density and anatomical properties. For healthy trees, a trunk wedge was sampled from the bark and sapwood at DBH. Wounds created on the trees were treated with fungicides and pesticides.

2.3. Wood density

From the first part of the disc, blocks of cm^3 were cut continuously (no interval) from the juvenile core towards the sapwood using a rotary saw. Oven dry wood densities of the samples were calculated based on ISO 3131- 1975 (E). Wood samples were first oven-dried at 103 ± 2°C to reach 0 % moisture content and weighed. Sample blocks were subsequently soaked in distilled water and block volumes obtained with accuracy of ±0.01 g. Prior to immersion, the samples were covered with a thin layer of paraffin wax. The wood density index (WD) was calculated as follows:

$$\rho = \frac{M}{V} = \frac{g}{cm^3} \qquad \text{(Equ. 1)}$$

Where ρ, M and V are for density, Mass and Volume, respectively.

2.4. Preparation for Macroscopic Measurement

The second part of each disc was prepared using series of sandpapers 80, 240, and 400 using an orbital sanding machine to reach a smooth, perfectly clear surface [19], before scanning at 2400 dpi resolution. Annual ring widths from 1987-2016 from each disc were measured radially from the pith to the most recent sapwood under a binocular microscope attached to the LINTAB 5 ring-width measuring system (Rinntech Company, Germany).

2.5. Sample Preparation for Sectioning

The disc was cut radially into blocks of 1 cm3, from bark to the pith at three positions: sapwood and bark, heartwood (middle), and near the pith. Each sample block contained approximately 10 growth rings. Blocks were labelled with site, dieback class, and their position at the disc. To better conserve the wood blocks, samples were fixed in FAA solution (50% ethanol: acetic acid: formaldehyde at 1:1:1, v: v: v). To soften the blocks and to improve section quality, samples were placed in an oven at 60 ºC for 24 hours.

2.5.1. Sectioning

A conventional method was used for sectioning. Cross sections with thickness of 20-25 µm were cut using a rotary microtome (POOYAN MK 1110, Iran). The sections were placed in sodium hypochlorite solution (10%) until they totally are bleached for about 5 minutes. The sections were then washed several times with distilled water until disappearing the bleaching odor. Sections were stained in 0.1 % (w/v) safranin O and washed twice in distilled water for about 5 minutes. Subsequently, sections were dehydrated in an ethanol series of 60%, 85%, 95% and absolute for 15 minutes. Dehydrated sections were fixed on slides using Canada balsam [20], prior to observation under an Olympus cx22LED microscope. Photographs of the sections were captured using a digital camera (True chrome metrics, China) attached to a computer. Mean tangential and radial diameters of earlywood vessels (DEV), earlywood vessels area (EVA), vessels density (number of vessels per mm^2, VD), and total number of tyloses (NT) visible in 30 standard observation fields of 500 μm^2 were measured from each decline class at each site.

2.6. Statistical Analysis

The data were subjected to one-way analysis of variance (ANOVA). Normality and homogeneity of the data were evaluated using Shapiro–Wilk's and Levene's tests, respectively. Independent sample t-tests were carried out to determine differences in wood properties among two healthy and declined trees at 1% and 5% levels of probability. The data were analyzed in SPSS software, version 21.

3. Results

3.1. Physical-anatomical variables

3.1.1. Wood density (WD)

There were significant overall differences between healthy and declined trees from the sampled sites ($P<0.01$; Table 2). The mean oven dry wood density (WD) of declined trees was higher than the WD in healthy trees (Table 3). A decreasing trend in WD from juvenile core to sapwood was found in declined at all three sites. Comparing healthy trees in sites B and C against declined trees, there was a noticeable reduction in WD in the juvenile core vs. the heartwood with no significant difference in the heartwood vs. in sapwood blocks taken near to the bark (Figure 3).

Table 2. One-Way ANOVA summarizing significance of wood-anatomical variables (WD: wood density; ARW: annual ring width) between healthy and declined *Quercus brantii* affected by drought stress in three oak forest sites in Iran.

			Mean square	
Source of Variation	**df**	**df**	**WD (g/cm^3)**	**ARW (µm)**
Decline	1	1	0.57^{**}	115.408^{**}
Sites	2	2	0.13^{**}	201.184^{**}
Decline × Sites	2	2	0.005^{*}	47.531^{**}
Error	174	90	0.002	1.439

Variables: WD: wood density; ARW: annual ring width; *Significance at 0.05; **Significance at 0.01.

Table 3. Summary of Student's t- test analyses of differences in wood characteristics (means ± SE) between healthy and declined *Quercus brantii* affected by drought stress.

Attributes	Sampling Group	Mean	Difference	*t*-test
WD (g/cm^3)	Declined	0.92±0.02	0.048	5.181^{*}
	Healthy	0.87±0.06		
ARW (µm)	Declined	0.78±0.24	1.60	5.241^{*}
	Healthy	2.38±0.54		

Variables: WD: wood density; ARW: annual ring width. (*): $p<0.01$.

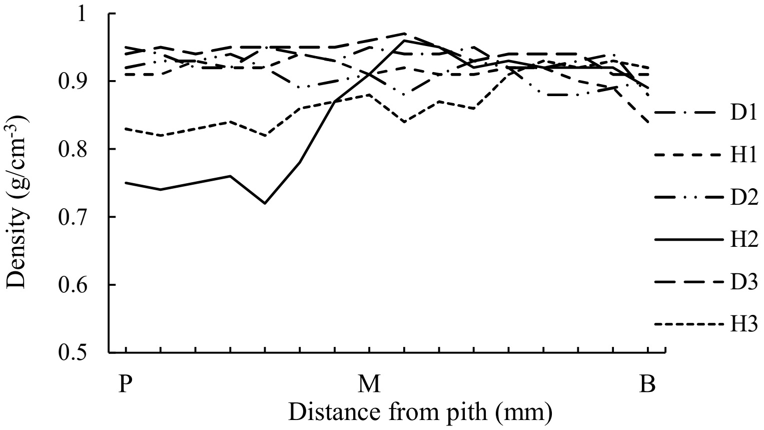

Figure 3. Wood density of declining (D1-D3) and healthy *Quercus brantii* (H1- H3) at three sites of Dareh shahr, Sheshdar and Chavar, Iran. Density was measured in the xylem of the juvenile core, heartwood, and sapwood, (H: Healthy, D: declined / 1: site 1, 2: site 2, 3: site 3, P: Pith, M: heartwood, B: sapwood).

3.1.2. Annual ring width (ARW)

Comparing ARW over time (Figures 4 and 5, Table 2), there was a significant difference ($P<0.01$) between means in declined compared with healthy trees across all three sites. ARW of declined trees at all sampling sites decreased over time, compared with healthy trees (Table 3). The decreases of ARW in declined trees were very similar at sites A and C. In healthy trees at both sites A and C, there was a similar trend between 2000 – 2016. Furthermore, in site B, the ARW decreased in declined trees compared to healthy trees (Figure 6).

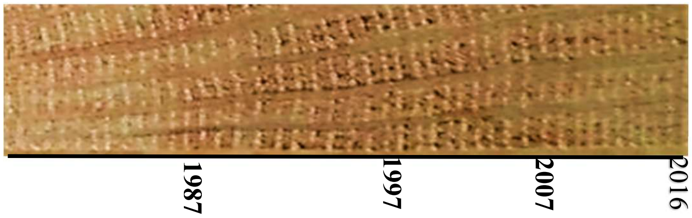

Figure 4. Transverse section of declined *Quercus brantii* showing reductions in annual growth ring width from the juvenile core near the pith towards the sapwood.

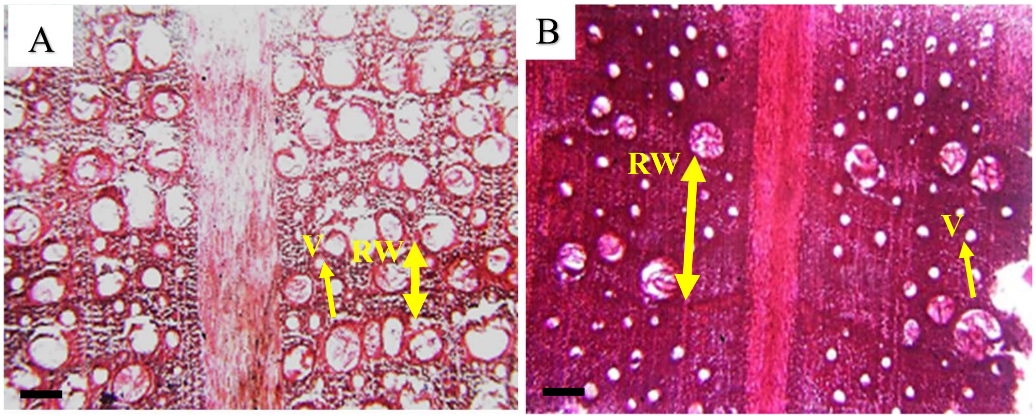

Figure 5. Variation in annual growth rings in transverse sectional view from *Quercus brantii*: (A), narrow rings with small vessels in trees showing symptoms of decline compared to (B) wider rings with larger vessels in healthy oaks (RW: ring width, V: vessels). Scale bars=75µm.

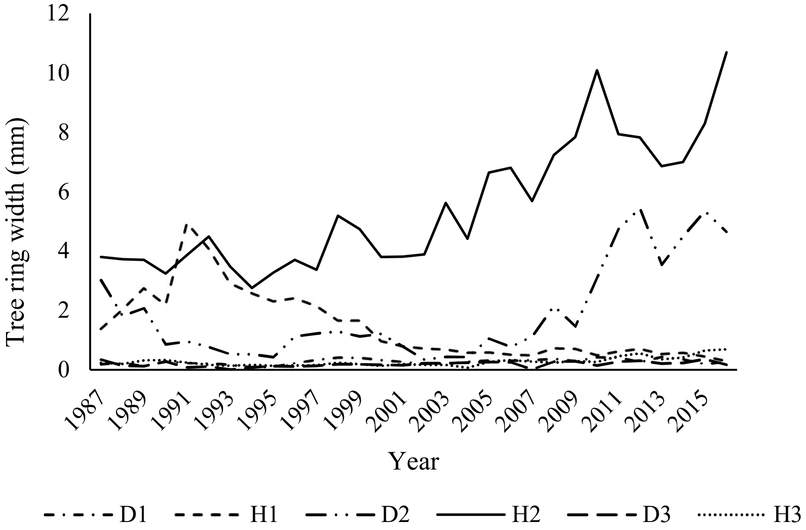

Figure 6. Time series of annual ring width of *Quercus brantii* with decline (D1- D3) and healthy trees (H1- H3).

3.2 Wood anatomy features

3.2.1. Diameter of earlywood vessels (DEV)

Variance analysis (Table 4) showed the effect of decline on DEV in three parts of the stem cross-section: juvenile core, heartwood and sapwood. Site also had a significant impact on DEV in the sapwood and heartwood. The interaction effects of decline and site were also significant in the three sampled parts of the trees. The mean DEV of declined trees showed a descending trend from the juvenile core to the bark at sites A and C, and was constant and similar in healthy trees. In the declined trees from site B, the trend in radial direction was constant and similar, while in healthy trees from site B there was a decreasing trend from juvenile core to sapwood (285.6 down to 132.1 µm, respectively) (Figures 7). In general, the mean DEVs in declined trees from all three sampled parts of the stems were lower than in healthy trees. There were significant differences between healthy and declined trees in the juvenile core, heartwood and sapwood (Table 5).

Table 4. One-Way ANOVA of wood-anatomical variables between healthy and trees with decline at the three study sites.

The Source of Variation	**df**	**DEV (µm)**	**EVA (µm)**	**VD (mm^{-1})**	**NT (mm^{-1})**
Decline J core	1	38.977**	2.742^{ns}	1.533^{ns}	0.182^{ns}
Heartwood		762.045**	127.558**	2.219^{ns}	8.477**
Sapwood		197.282**	64.223**	64.335**	25.104**
Sites J core	2	1.571^{ns}	1.014^{ns}	3.633*	14.871**
Heartwood		9.400**	3.984*	6.040**	96.150**
Sapwood		67.933**	4.442*	10.347**	99.973**
Decline × Sites J core	2	13.842**	1.440^{ns}	0.722^{ns}	3.553*
Heartwood		32.288**	1.330^{ns}	0.367^{ns}	2.898^{ns}
Sapwood		258.509**	4.221*	14.944**	7.412**
Error J core	594	12267.234	2.622E+11	2.010	0.918
Heartwood		2808.444	2165248861	1.730	1.387
Sapwood		3290.675	4289042107	2.104	1.396

J core: juvenile core. Variables: DEV: diameter of earlywood vessels; EVA: earlywood vessels area; VD: vessels density; NT: number of tyloses; *Significance at 0.05; **Significance at 0.01; ns: non- significant.

Table 5. Differences in wood characteristics (means ± SE) between healthy and trees with decline based on the Student's *t*-test.

Anatomical features	**Sampling Group**	**Juvenile core**	**Heartwood**	**Sapwood**
DEV (µm)	Declined	228.43±15.54**	171.74±6.72**	173.85±7.9**
	Healthy	284.88±10	291.19±6.28	239.64±10.96
EVA (µm)	Declined	38754.64±3.391.46^{ns}	21669.15±2.852.86**	22720.51±2.646.88**
	Healthy	10.7979.78±83.605.98	107979.78±7.088.08	65573.28±10.484.12
VD (mm^{-1})	Declined	1.62±0.16^{ns}	2.09±0.16^{ns}	2.24±0.18**
	Healthy	1.48±0.14	1.93±0.12	1.29±0.14
NT (mm^{-1})	Declined	0.67±0.12^{ns}	1.32±0.16^{ns}	1.15±0.16**
	Healthy	0.64±0.08	1.04±0.12	0.66±0.14

Variables: DEV: diameter of earlywood vessels; EVA: earlywood vessels area; VD: vessels density; NT: number of tyloses. (**): $p<0.01$/ns: non- significant.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 April 2021

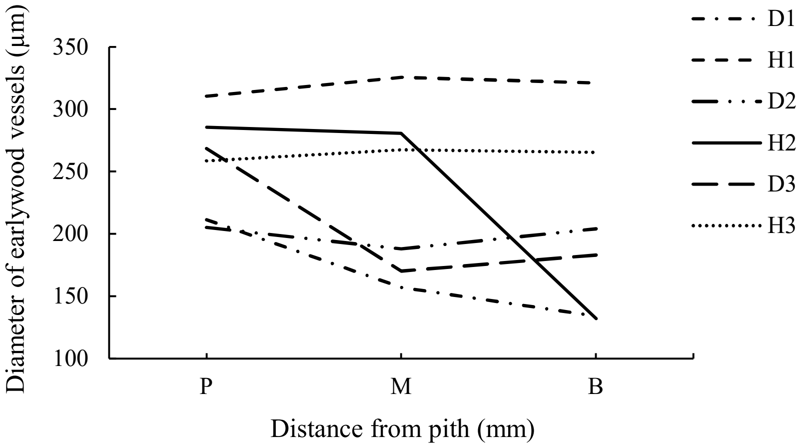

Figure 7. Diameter of earlywood vessels in declined and healthy trees of *Quercus brantii* in the juvenile core surrounding the pith (P); heartwood (M), and sapwood (B).

3.2.2. Earlywood vessels area (EVA)

Decline and site had significant effects on EVA in the heartwood and sapwood, with no significant differences in the juvenile core. In addition, interaction effects of decline × site were significant in sapwood, but not significant in the heartwood or juvenile core (Table 4). The mean EVA of declined trees declined trend from juvenile core to sapwood at all three sites. Healthy trees in sites A and B showed a decreasing trend from juvenile core to sapwood, while an increasing trend was observed at site C (53816.468 to 82628.982 µm, respectively) (Figure 8). In general, the mean EVA in declined trees from the three sampling positions in stems: juvenile core, heartwood and sapwood was lower than in healthy trees. Significant differences in EVA were found between healthy and declined trees in the heartwood and sapwood (Table 5).

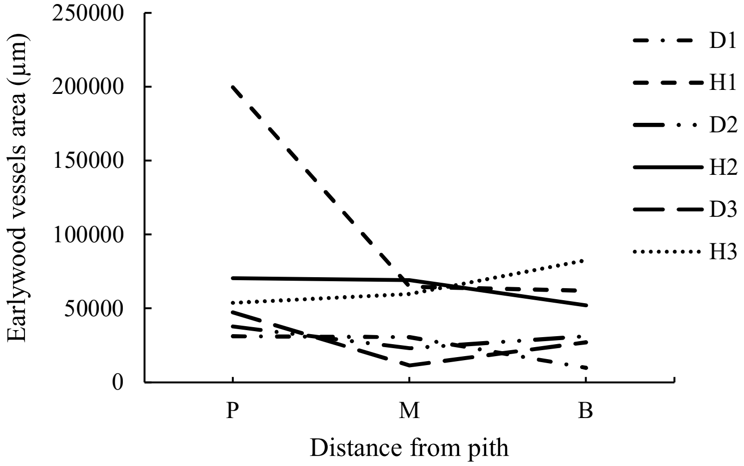

Figure 8. Comparisons of area of eearlywood vessels in declined and healthy trees of *Quercus brantii* in juvenile core near the pith, heartwood and sapwood of stems.

3.2.3. Vessels density (VD)

Decline had a significant effect on VD only in current sapwood (Table 4). Site, however, had a significant effect on VD in all three sampling positions. There was no significant interaction effect of decline × site in the heartwood and juvenile core tissues. The highest VD was in the sapwood in declined trees from all three sites (Figure 5A). In the healthy trees, an increasing trend in VD occurred from juvenile core to sapwood at site A (1.3 to 1.62 mm^{-1}, respectively). In contrast, healthy trees of site B had a decreasing trend in VD from juvenile core to sapwood (1.39 to 0.5 mm^{-1}, respectively). However, healthy trees at site C showed a fixed trend from juvenile core to sapwood (Figure 9). In general, VD in declined trees from the three tissue types sampled were higher than in healthy trees. Finally, significant differences in VD were observed between healthy and declined trees only in sapwood (Table 5).

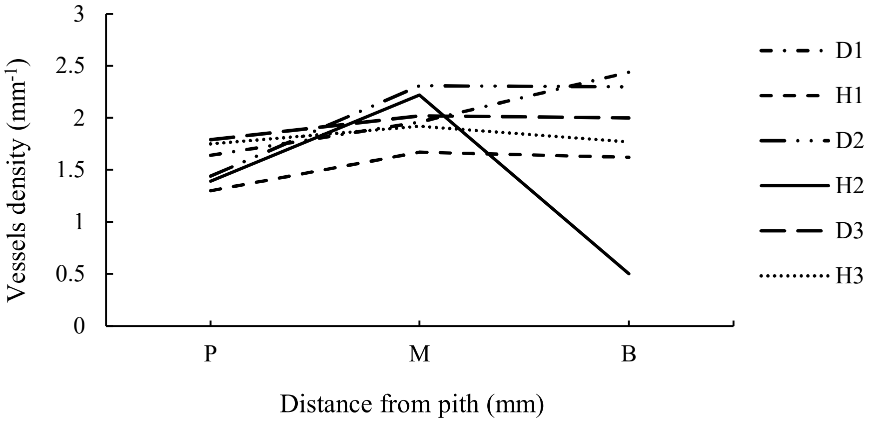

Figure 9. Vessels density in declined and healthy *Quercus brantii* in juvenile core, heartwood and sapwood of stems.

3.2.4. Number of tyloses (NT)

The effect of decline on the NT was significant in vessels in the heartwood and sapwood of stem sections (Table 4). NT varied significantly between the three sites in all sample tissues. Interaction effects, however, differed significantly only in the juvenile core and sapwood. In declined trees, mean NT showed an increasing trend from juvenile core to sapwood at all three sites. Healthy trees in sites A and B had the lowest NT in sapwood, with an increase towards the pith. Furthermore, NT in healthy trees showed an increasing trend from juvenile core to sapwood in trees at site C (0.65 to 1.8 mm^{-1}) (Figures 10 and 11). Thus, higher NTs were found in declined trees from the juvenile core, heartwood and sapwood compared with healthy trees. Finally, there were significant differences between NT in healthy and declined trees in sapwood, with no significant differences between the heartwood and juvenile core vessels (Table 5).

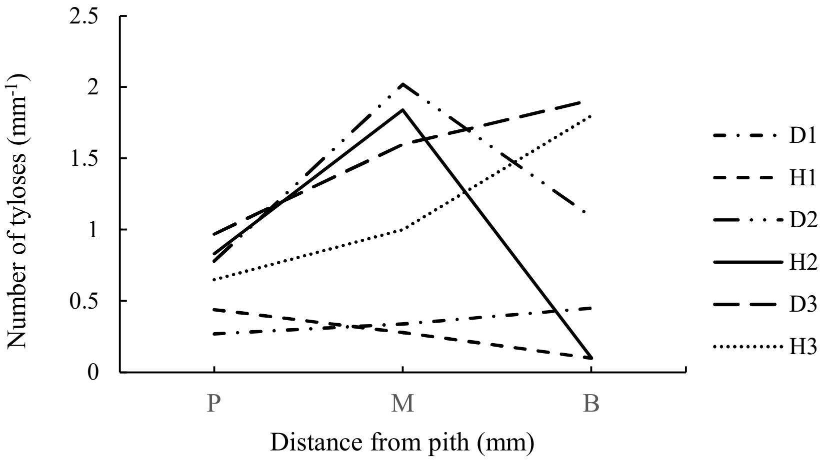

Figure 10. Number of tyloses in xylem vessels of declining (D) or healthy (H) *Quercus brantii* in the juvenile core, heartwood and sapwood.

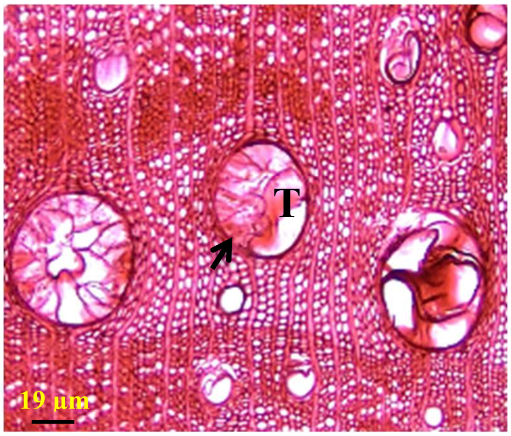

Figure 11. Tyloses (T) occluding vessel lumen in transverse section of a declined tree from a sapwood part.

4. Discussion

In this work, variations in annual growth ring and vessel sizes, and other wood anatomical features were investigated in *Quercus brantii* affected by drought stress. In general, increases in VD and NT led to increased WD in declined trees, agreeing with previously published work on oak showing that wood density varies as vessel features change [21]. Santiago et al. [22] indicated that an increase in total surface area of the vessels leads to lower wood density. The occurrence of vascular anomalies as reactions to mechanical or physiological disturbance emphasizes the crucial importance of xylem vessels in tree physiology, because it is thought to play a role in a trading triangle comprising three competing functions: water transport, mechanical support and resistance to embolisms [23].

Density is an important attribute in wood quality, and is associated with other determinative properties. Wood density mainly varies through differences in anatomical properties, such as size and distribution of different cells [24], and is strongly related to cell size and vessel proportions [25, 26]. In the work on *Q. brantii* presented here, DEV and EVA of declined trees in the three sampled sites showed a descending trend from juvenile core to the sapwood. Due to the decrease in the DEV and EVA and increase in the VD in the wood in the sapwood of trees with decline, it appeared that vessel number and density were inversely correlated with average vessel area [27]. It is also known that an increased total surface area of vessels leads to lower wood density [22].

Annual rings are an important source of environmental information [28], enabling variations in climate to be tracked over thousands of years. Narrow rings indicate undesirable environmental conditions (drought or cold periods), whereas wide rings suggest good growth in reasonable conditions (warmer or wetter). In this work, ARW of trees suffering from decline was significantly smaller than in healthy trees. Although the main driver of this decline in ARW is probably the drought suffered by the trees over a protracted time, it is also possible that changes in life strategies of endophytic fungi present naturally in the plant tissues, becoming pathogenic under conditions stressing to the host, may further weaken the host plant [29]. Average DEV and EVA in declined trees was smaller than the same features in healthy *Q. brantii*. Decline due to drought could lead to higher VDs in affected trees, as the plants attempt to improve sap flow to the photosynthetic tissues in the crown. Simultaneous reduction of DEV and EVA will lead to a reduction in sap flow in the trees, especially those in decline. Thus, adverse changes in certain wood structural parameters such as vessel diameters are important for hydraulic conductivity. The changes may indicate that the tree genotypes most badly affected are poorly adapted to changing environmental conditions and will, therefore, begin to decline. Furthermore, in response to environmental changes, trees can regulate water conductance further by changing the size and density of the vessels [30, 31]. Radial growth of declining *Q. robur* in Poland showed that the average tree ring width and the diameter of earlywood vessels in trees that had died was lower than in healthy oaks [32].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 April 2021

Water conductance to leaves in ring-porous trees, such as oaks, is highly dependent on large, functional earlywood vessels. Usually, there is an inverse relationship between the size and the number of vessels, therefore trees will optimize or increase hydraulic conductivity according to environmental conditions [33]. In trees affected by increasing environmental stresses, the diameter of earlywood vessels and, as a consequence, the hydraulic conductivity decreases [34]. However, trees may form high numbers of vessels in an attempt to compensate for the problem [35, 36]. Likewise, small vessel diameter is highly dependent on climate conditions, including drought [37, 38].

Oaks usually have large and wide vessels, particularly in the earlywood, and embolism is relatively frequent [39]. Compromising the vascular system when invaded by a pathogen leads to blocking of vessels by expansion of tyloses from xylem companion cells, sometimes with secretion of gums and gels to isolate the damaged tissues. Formation of tyloses is an important cause of vascular blockage, preventing sap flow. When the process occurs excessively, it can be a major cause of dieback in the plant. In this study, NT in declined trees at all three sites increased in sapwood vessels, compared to xylem in the juvenile core near the pith. NT was directly related to VD and inversely related to DEV, EVA. Furthermore, there were significant differences in NT and VD in the sapwood of declined trees, but no significant differences in these features in the xylem in the juvenile core of trees in decline. It is probable, therefore, that trees which eventually died from decline probably had the greatest weakness in hydraulic conductivity due to reductions in DEV and EVA, and increases in NT and VD. It is well known that anatomical variations caused by abiotic stress decrease with increasing distance from the affected area (here, sapwood towards juvenile core) [40].

The presence of tyloses in earlywood vessels of the annual wood increment has an influence on the reduction of hydraulic conductance in declining oaks [32]. Earlywood vessels contribute to large amounts of sap flow in the main stem and along branches, but are vulnerable to embolization [41]. Decreased lumen diameters in severely stressed or infected oak may assist in controlling water transport and reducing vulnerability to cavitation and embolism [42].

Large-scale and local climate change will have many different effects on individual trees, forest structure and growth performance, including the development and prevalence of fungal diseases [43]. It is likely that the increasing occurrence of charcoal disease, caused by *Biscogniauxia* *mediterranea* in oaks in the Zagros Mountains is related to recent drought phenomena, emphasizing the need to examine in depth the relationship between abiotic climatic and biotic factors [7]. Many biotic and abiotic factors are certainly involved in the decline of oak trees, leading Henrique et al. [44] to suggest the complexities interacting factors determining the roles of different factors in the survival of this tree.

5. Conclusions

Recent studies have not provided comprehensive evidence quantifying the impact of drought stress on Persian oak anatomical features and wood density. The results have given an account of the disorder from an abiotic stress on the wood density, growth ring width, diameter of vessels, vessels area, vessel and tyloses number. Noticeable variation in ARW, DEV, EVA, NT and VD at different sites from juvenile core to sapwood of *Q. brantii* in decline were observed. The WD of declined trees from the juvenile core to sapwood showed decreasing trend. Water conduction would decrease in response to vessel size and increased VD in declined trees from the juvenile core to sapwood, which contributes to loss of viability and ultimately death of trees. The results of this study also provide a theoretical basis for future related research on hydraulic parameters in declined trees.

Acknowledgments

We would like to thank Miss. A. Hawasi and Dr. S. Soltani for their kind help during this work.

Author Contributions:

F.S. and H.R.N. carried out the experiment and wrote the manuscript with support from S.W.; I.A. performed the computations and verified the analytical methods. H.A.H helped supervise the project. All authors contributed to the final manuscript.

Funding:

This research did not receive any specific funding.

Conflicts of Interest:

The authors declare no conflicts of interest

References

1. Colangelo, M.; Camarero, J.J.; Borghetti, M.; Gentilesca, T.; Oliva, J.; Redondo, M.A. et al. Drought and Phytophthora are associated with the decline of oak species in southern Italy. *Front Plant Sci.* **2018,** *9*, 1595.

2. Kabrick, J.M.; Dey, D.C.; Randy, R.G.; Wallendorf, M. The role of environmental factors in oak decline and mortality in the Ozark Highlands. *For Ecol Manag* **2008,** *255,* 1409-17.

3. Philip, M.; David, R.; Leon, A. Oak decline. Forest Insect and Disease Leaflet. **1983,** *165*, 1-8.

4. Nourinejad, J.; Rostami, A. Investigation of oak decline and its relation to physiographic factors in the forests of West of Iran. *JBES* **2014,** *5,* 201-207.

5. Sepahvand, T.; Zandebasiri, M. 2014. Evaluation of Oak decline with local resident, opinions in Zagros forests, Iran. *SJAS* **2014,** 4(4), 231-234.

6. Evert, R F.; Eichhorn, S.E. Esau's Plant Anatomy: Meristems, Cells, and Tissues of the Plant Body: Their Structure, Function, and Development. Hoboken, NJ: John Wiley and Sons, Inc. **2006.**

7. Sturrock, R.N.; Frankel, S.J.; Brown, A.V.; Hennon, P.E.; Kliejunas, J.T.; Lewis, K.T. et al. Climate change and forest diseases. *Plant Pathol* **2011,** *60*, 133-149.

8. Pedersen, B.S. The role of stress in the mortality of midwestern oaks as indicated by growth prior to death. *Ecology* **1998a,** *79,* 79-93.

9. Kopabayeva, A.; Mazarzhanova, K.; Köse, N.; Akkemik, U. Tree-ring chronologies of *Pinus sylvestris* from Burabai Region (Kazakhstan) and their response to climate change. *Dendrobiology* **2017,** *78*, 96-110.

10. Agusti, J.; Greb, T. Going with the wind adaptive dynamics of plant secondary meristems. *Mechanisms of Development* **2013,** *130*, 34-44.

11. Balducci, L.; Cuny, H.E.; Rathgeber, C.B.K.; Deslauriers, A.; Giovannelli, A.; Rossi, S. Compensatory mechanisms mitigate the effect of warming and drought on wood formation. *Plant Cell Environ* **2016,** *39*, 1338-1352.

12. Cuny, H.E.; Rathgeber, C.B.K.; Kiess´e, T.S.; Hartmann, F.P.; Barbeito, I.; Fournier, M. Generalized additive models reveal the intrinsic complexity of wood formation dynamics. *J. Exp. Bot.* **2013,** *64*,1983–1994.

13. Brodribb, T.J.; Cochard, H. Hydraulic failure defines the recovery and point of death inwater-stressed conifers. *Plant Physiol* 2009, *149,* 575–584.

14. Solla, A.; Gil, L. Xylem vessel diameter as factor in resistance of *Ulmus minor* to *Ophiostoma novo-ulmi*. *For Pathol* **2002**, *32*, 123-134.

15. Anderegg, W.R.L.; Hicke, J.A.; Fisher, R.A.; Allen, C.D.; Aukema, J.; Bentz, B.; Hood, S.; Lichstein J.W.; Macalady, A.K.; McDowell, N.; Pan, Y.; Raffa, K.; Sala, A.; Shaw, J.D.; Stephenson, N.L.; Tague, C.; Zeppel, M. Tree mortality from drought, insects, and their interactions in a changing climate. *New Phytol* **2015,** *208*, 674‑683.

16. Giagli, K.; Gričar, J.; Vavrčik, H.; Menšik, L.; Gryc, V. The effects of drought on wood formation in *Fagus sylvatica* during two contrasting years. *IAWA Journal* **2016,** *37*, 332‑348.

17. Naji, H.R.; Taher Pour, M. The effect of simulated dust storm on wood development and leaf stomata in *Quercus brantii* L. *Desert.* **2019,** *24,* 43-49.

18. Aloni, R..; Zimmermann, M.H.Z. The control of vessel size and density along the plant axis. A new hypothesis. Differentiation **1983,** *24*, 203-208.

ISO 3131. (1975). *Wood-Determination of Density for Physical and Mechanical Tests.* International Organization for Standardization, Geneva.

19. Radmehr, A.; Soosani, J.; Balapour, Sh.; Hosseini Ghale Bahmani, S.M. Effects of climate variables (temperature and precipitation) on the width of Rings-growth in Persian coppice oak in the central Zagros (Case study: Khoramabad), *Journal of Wood & Forest Science and Technology* **2014,** *22*, 93-110. (In Persian).

20. Camargo, M.A.B.; Marenco, R.A. Density, size and distribution of stomata in 35 rainforest tree species in Central Amazonia. *Acta Amaz* **2011,** *41*, 205-212.

21. Rao, R.V.; Aebischer, D.P.; Denne, M.P. Latewood density in relation to wood fibre diameter, wall thickness, and fibre and vessel percentages in *Quercus robur* L. *IAWA Journal* **1997,** *18*, 127-138.

22. Santiago, L.S.; Goldstein, G.; Meinzer, F.C.; Fisher, J.B.; Machado, K.; Woodruff, D.; Jones, T. Leaf photosynthetic traits scale with hydraulic conductivity and wood density in Panamian forest canopy trees. *Ecophysiology* **2004,** *140*, 543–550.

23. Baas, P.; Ewers, F.W.; Davis, S.D.; Wheeler, E.A. Evolution of xylem physiology. In: Hemsley AR, Poole I, editors. The Evolution of Plant Physiology. London: *Elsevier Academic Press*, **2004,** 273-295.

24. Zobel, B.J.; Van Buijtenen, J.P. Wood variation, its causes and control. Springer-Verlag, Berlin, Germany, **1989,** 363.

25. Preston, K.A.; Cornwell, W.K.; DeNoyer, J.L. Wood density and vessel traits as distinct correlates of ecological strategy in 51 California coast range angiosperms. *New Phytol* **2006,** *170*, 807-818.

26. Roque, R.M.; Tomazello-Filho, M. Relationships between anatomical features and intra-ring wood density profiles in Gmelina arborea applying X-ray densitometry. *Cerne,* **2007,** *13*, 384–392.

27. Pourtahmasi, K.; Lotfiomaran, N.; Brauning, A.; Parsapajouh, D. Tree-ring width and vessel characteristics of oriental beech (*Fagus orientalis*) along an altitudinal gradient in the Caspian forests, Northern Iran. *IAWA Journal* **2011,** *32*, 461- 473.

28. Fritts, H.C. Tree ring and climate. Academic Press. London, 1976, 567.

29. Mirabolfathy, M.; Groenewald, J.; Crous, P. The occurrence of charcoal disease caused by *Biscogniauxia mediterranea* on chestnut-leaved oak (*Quercus castaneifolia*) in the Golestan Forests of Iran. *Plant Dis* **2013,** *95*, 3539.

30. Reyes-Santamarı´a I.; Terrazas, T.; Barrientos-Priego, A.F.; Trejo, C. Xylem conductivity and vulnerability in cultivars and races of avocado. *Sci Hortic* **2002,** *92*, 97-105.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 April 2021

31. Bayramzadeh, V.; Funada, R.; Kubo, T. Relationships between vessel element anatomy and physiological as well as morphological traits of leaves in Fagus crenata seedlings originating from different provenances. *Trees* **2008,** *22*, 217-224.

32. Tulik, M. The anatomical traits of trunk wood and their relevance to oak (*Quercus robur* L.) vitality. *Eur. J. For. Res* **2014,** *133*(5), 845-855.

33. Oladi, R.; Bräuning, A.; Pourtahmasi, K. Plastic" and "static" behavior of vessel-anatomical features in Oriental beech (*Fagus orientalis* Lipsky) in view of xylem hydraulic conductivity. *Trees* **2014,** *28*, 493-502.

34. Levanic, T.; Cater, M.; McDowell, N.G. Associations between growth, wood anatomy, carbon isotope discrimination and mortality in a *Quercus robur* forest. *Tree Physio*l **2011,** *31*(3), 298-308.

35. Nabeshima, E.; Kubo, T.; Yasue, K.; Hiura, T.; Funada, R. Changes in radial growth of earlywood in *Quercus crispula* between 1970 and 2004 reflect climate change. *Trees* **2015,** *29*, 1273-1281.

36. Oladi, R.; Nasiriani, S.; Danekar, A.; Pourtahmasi, K. Inter-relations between tree-ring width and vessel features in black alder (*Alnus glutinosa*). *Iranian Journal of Wood and Paper Science* **2016,** *30*, 278-288.

37. Kulkarni, M.; Schneider, B.; Raveh, E.; Tel-Zur, A.N. Leaf anatomical characteristics and physiological responses to short-term drought in Ziziphus mauritiana (Lamk.). *Sci Hortic* **2010,** *124*, 316-322.

38. Plavcova, L.; Hacke, U.G. Phenotypic and developmental plasticity of xylem in hybrid poplar saplings subjected to experimental drought, nitrogen fertilization, and shading. *J. Exp. Bot* **2012,** *63*, 6481-6491.

39. Sperry, J.S.; Nichols, K L.; Sullivan, J.E.; M.; Eastlack, S.E. Xylem embolism in ring-porous, diffuse-porous, and coniferous trees of northern Utah and interior Alaska. *Ecology* **1994,** *75*, 1736-1752.

40. Fink, S. Pathological and Regenerative Plant Anatomy. Encyclopedia of Plant Anatomy, Volume 14, Part 6. Berlin: *Gebrüder Borntraeger* **1999,** 1095.

41. Tyree, M.T.; Zimmermann, M.H. Xylem structure and the ascent of sap. 2ndEdition. Berlin: Springer-Verlag. **2002.**

42. Cochard, H.; Peiffer, M.; Le Gall, K.; Granier, A. Developmental control of xylem hydraulic resistances and vulnerability to embolism in *Fraxinus excelsior* L.: impacts of water relations. *Journal* of *Experimental Botany* **1997,** *48*, 655-663.

43. Baguskas, S.A.; Peterson, S.H.; Bookhagen, B.; Still, C.J. Evaluating spatial patterns of droughtinduced tree mortality in a coastal California pine forest. *Forest Ecol and Manag* **2014,** 315, 43– 53.

44. Henriques, J.; Barrento, M.J.; Bonifácio, L.; Gomes, A.A. Lima, A.; Sousa, E. Factors affecting the dispersion of *Biscogniauxia mediterranea* in Portuguese cork oak stands. *Silva Lusitanica* **2014,** *22*, 83-97.