Research Article
doi: 10.1590/01047760202228013090
vol(28), 2022

CERNE

Differential of three fields grown *Juniperus* species summer drought and cold hardening

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ECOLOGY

ABSTRACT

Background: Understanding species' reactions to environmental stressors (cold and drought) and characterizing drought tolerance can help us understand their ecosystem responses related to global change. This study aimed to understand and compare the drought and cold tolerance strategies of *Juniperus excelsa*, *Juniperus foetidissima*, and *Juniperus oxycedrus*, which are found in the Western Mediterranean Region. Water relation parameters [Ψ_{TLP} (osmotic potential at turgor loss point), Ψ_{100} (osmotic potential at full turgor), εmax (bulk modulus of elasticity), V0/DW (symplastic water at the saturated point per dry weight of the shoot), RWC (relative water content)] in summer and winter were determined. Total soluble sugar and photosynthetic pigment content were identified in spring, summer, autumn, and winter.

Results: *J. foetidissima* had lower Ψ_{TLP} and higher εmax in summer than other species. The species had similar Ψ_{TLP}, Ψ_{100}, εmax, V0/DW, and RWC in winter. A seasonal change was observed in total soluble sugar and photosynthetic pigment content. Total soluble sugar and photosynthetic pigment contents were related to mean air temperature and total precipitation.

Conclusion: *J. foetidissima* was more tolerant to water deficit in the summer, whereas the three species reacted similarly to the cold in the winter.

Keywords: Drought, water status, cold resistance, soluble sugar, *Juniperus*, Mediterranean

HIGHLIGHTS

The water relations of *Juniperus* species were generally different in summer but similar in winter.
All three *Juniperus* species had lower total soluble sugar content in summer than in winter.
While photosynthetic pigment content was high in all three species in summer, it decreased in winter.
J. foetidissima was more tolerant to water deficit in summer than *J. excelsa* and *J. oxycedrus*.

DELİGÖZ, A; BAYAR, E. Differential of three fields grown *Juniperus* species summer drought and cold hardening. CERNE, v.28, n.1, e-103090, doi:10.1590/01047760202228013090

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CERNE
ISSN 0104-7760
UNIVERSIDADE FEDERAL DE LAVRAS

INTRODUCTION

Arid and semi-arid regions constitute about 30% of the world's land surfaces (Sène, 2004). Cold and drought are critical environmental stressors that affect plants' growth, productivity, and worldwide distribution (Levitt, 1980). There is a large body of research on how species react to cold and drought (Kubiske and Abrams, 1991; Ouyang et al., 2019). In general, plant production in the Mediterranean climate is limited by low temperatures and low radiation in winter and high water stress and high temperatures in summer (Oliveria et al., 1992). Plants in regions with a Mediterranean climate have to cope with both long summer drought and short-term water stress due to high air temperature and evaporation (Correia et al., 2001). When plants are gradually exposed to adverse growing conditions and environmental stressors, they make physiological and biochemical adjustments to prevent damage (Scholz et al., 2012). At temperatures below freezing point, plants can survive either by avoiding or tolerating freezing (Levitt, 1980). Trees use similar strategies (including avoidance and tolerance) to deal with stress (Charrier et al., 2015). While the energy required for biochemical events increases, photosynthesis, carbohydrate transport, and respiration rates decrease at low temperatures (Lambers et al., 2008). While water stress causes a reduction in osmotic potential at turgor loss point and osmotic potential at full turgor, it leads to an increase in the modulus of elasticity (Pita and Pardos, 2001), and a change in photosynthetic pigment content (Michelozzi et al., 1995). Ψ_{TLP} indicates the irreversible level of cell water losses and constitutes the limit where death or drying begins (Genç and Yahyaoğlu, 2007). It is generally determined using pressure-volume (PV) curves (Tyree and Richter, 1982). ε indicates how much turgor potential decreases during leaf water losses (Saito et al., 2006). Elastic adaptation plays a critical role as a drought tolerance mechanism (Bacelar et al., 2009). Characteristics related to plant-water relationships, such as Ψ_{TLP}, Ψ_{100}, and εmax, are important parameters for assessing plants' drought tolerance and adaptation (Bartlett et al., 2012; Zhu et al., 2018). Species with more negative water potential (i.e., turgor loss) are generally more drought tolerant (Bartlett et al., 2016; Kunert, 2020; Kunert et al., 2021).

Drought avoidance and tolerance determine the physiological effects of drought stress on a species and its distribution in habitats with varying soil water availability (Parker et al., 1982). A plant's capacity to respond to stressors affects its geographic distribution. A significant part of the forest tree species in Türkiye is distributed in environments with summer drought and Mediterranean climatic conditions (Dirik, 1994). Juniper species, known for their drought resistance, cover an area of 1 472 988 ha in Türkiye (OGM, 2020). Due to their aesthetic body forms, junipers are ornamental trees (park and garden arrangements). They are also used in erosion control because they are resistant to extreme climate and soil conditions and have widespread root systems. They are valuable assets for the wood-based industry. They are also versatile wood species used in wind, snow, and sound curtains (Gültekin et al., 2003). *Juniperus excelsa* Bieb. is found in stands in large areas in the forests of Türkiye. It stretches from marine climate zones to the steppe. It is resistant to heat, cold, and drought (Eler, 2000). Durable and valuable wood is obtained from *Juniperus foetidissima* Willd. *Juniperus oxycedrus* L. can grow almost anywhere (Anşin and Özkan, 2006). *Juniperus* species can adapt to extremely harsh environmental conditions (summer drought, winter frosts, shallow soil, etc.) that no other tree species can survive (Douaihy et al., 2013). According to ecophysiological studies, different genotypes have different drought coping capacities, (Villar et al., 2011) and different species have different levels of cold tolerance (Charra-Vaskou et al., 2012). Climate change requires trees and forests to cope with new climatic and biotic conditions. Tree populations cope with new climatic conditions by either migrating or adapting. If they cannot cope, they vanish. We should understand the adaptation mechanisms of forests and trees to address their capacity to survive and thrive (Chmura et al., 2011). This study had two objectives: (a) understanding and comparing the drought and cold tolerance strategies of *J. foetidissima*, *J. excelsa* and *J. oxycedrus* species under natural environmental conditions and (b) to determinining the seasonal changes in the osmotic potential at turgor loss point (Ψ_{TLP}), osmotic potential at full turgor (Ψ_{100}), bulk modulus of elasticity (εmax) , symplastic water at a saturated point per dry weight of the shoot (V0/DW), relative water content (RWC), soluble sugar, and photosynthetic pigment content.

MATERIAL AND METHODS

Field site and plants

The experimental plot is located Western Mediterranean Region in Türkiye (Isparta Regional Directorate of Forestry, Sütçüler Forestry Management Directorate 37°38′43″ N, 31°00′20″ E; 1366 m). It has a slope of 30% in the southeast. The study was conducted between April 2016 and January 2017. According to the data received from the nearest meteorological station, the experimental plot had a total precipitation rate of 880 mm, an average air temperature of 13.7 °C, and average air humidity of 58.0% between 2016 and 2017 (Table 1; Figure 1).

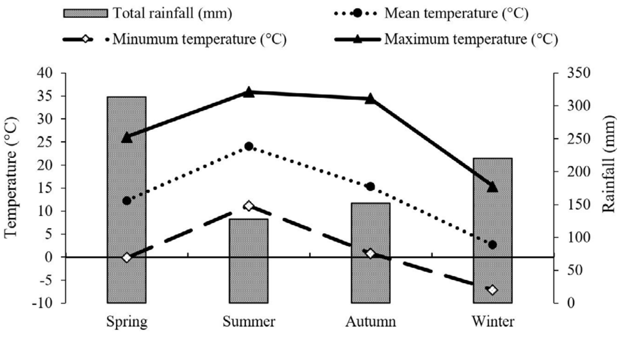

Figure 1. Total rainfall and minumum, mean and maximum air temperature at the experimental plot during the study period (2016-2017 years).

Table 1. Monthly rainfall and air temperature at the experimental plot during 1961–1992, 2007-2017 and 2016-2017 for the year.

	Months												Annual
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	
Rainfall 1961-1992 (mm)	141.4	129.3	91.6	86.6	63.7	35.3	11.3	11.8	32.6	68.3	101.4	163.8	937.1
Rainfall 2007-2017 (mm)	146.9	110.1	90.5	55.5	73.3	39.5	6.0	11.6	36.9	94.1	82.5	140.8	887.6
Rainfall 2016-2017 (mm)	166.6	38.1	101.0	47.2	129.7	88.4	10.2	16.2	22.6	25.9	141.0	93.1	880.0
Temperature 1961-1992 (°C)	3.4	3.9	7.0	11.3	15.9	20.6	24.3	24.0	20.0	14.7	9.1	5.0	13.3
Temperature 2007-2017 (°C)	3.7	5.0	7.8	11.8	16.1	21.2	25.3	25.5	21.1	15.2	10.2	5.9	14.1
Temperature 2016-2017 (°C)	1.9	6.0	8.0	12.7	14.9	21.1	25.6	24.4	20.9	15.5	9.0	4.6	13.7

The hottest months (average 25.0 °C) were July and August, and the coldest (average 1.2 °C) was January during the study period. The total precipitation was 36.2 mm in April, 7.6 mm in August, 0.2 mm in October, and 148.6 mm in January (Fig. 1). The experimental plot is semi-moist according to the Erinc method based on the long-term averages of climate data (Table 1). *J. excelsa* had mean diameter at breast height (cm) and mean height (m), 35.7±0.9 and 12.0±0.6, respectively. *J. foetidissima* had an average breast height diameter (cm) of 36.8±0.8 and a height (m) of 12.9±0.5. *J. oxycedrus* had an average breast height diameter (cm) of 16.1±0.8 and a height (m) of 3.6±0.4. The experimental plot was 1 ha in size.

Pressure volume analyses

Water relation parameters were examined in the summer (August) and winter (January). Shoot samples were collected from the south side of the crown and the lower 2/3 of the ten sample trees from each species. Shoots (15-20 cm) were collected from *J. foetidissima*, *J. excelsa*, and *J. oxycedrus*. Water potential Water relation parameters were detected on three randomly selected shoot samples. The shoots were immediately put in plastic bags and placed in a mini-fridge with ice sockets inside. They were then brought back to the laboratory. The Pressure-Volume (P-V) curve method was used to determine the water relation parameters. Measurements were made using a plant chamber device (PMS Instrument Co., Corvalis, OR, USA) (Scholander et al., 1965). They were cleaned under water and dried. Then their fresh weights (FW) (at 0.001 g sensitivity) were determined. Afterward, they were placed in distilled water and kept in the dark at room temperature for 24 hours. Their saturation weights (SW) were identified. They were then immediately placed in a plant pressure chamber device. They were kept for 10 minutes with an increase in pressure steps of 0.3 MPa (Ritchie, 1984) The measurement continued until the water potential became -4.0 or -4.5 MPa. Afterward, their end of measure weights were determined. They were then kept in a drying oven at 105 °C for 24 hours, and their oven-dried weight (DW) was then identified. Ψ_{TLP}, Ψ_{100}, and symplastic water content are determined using pressure-volume curves, while RWC and Ɛmax are calculated using the following equations: (Parker and Pallardy, 1988; Gross and Koch, 1991; Colombo and Teng, 1992; Mitchell et al., 2008; İmal, 2015).Where Ψ_{P1} and Ψ_{P2} are turgor pressures determined at 3-5 % intervals of RWC, V_1 and V_2 are symplast water volume at Ψ_{P1} and Ψ_{P2}, respectively. V is total symplast volume at full turgor.

$$RWC= [(FW-DW)/(SW-DW)]*100$$

$$\varepsilon max = (\Psi_{P1}-\Psi_{P2})/[(V_1-V_2)/V]$$

Photosynthetic pigment and total soluble sugar

Ten shoots were collected from each species (*J. foetidissima*, *J. excelsa* and *J. oxycedrus*) randomly selected in the experimental plot. Soluble sugar and photosynthetic pigment analyses were performed on the same trees using shoots collected for the P-V curve. Total soluble sugar and photosynthetic pigment content were determined in needles in the spring (April), summer (August), autumn (October), and winter (January). The chlorophyll pigment content was determined on fresh samples according to Arnon (1949). Measurements were made in a spectrophotometer at 450, 645, and 663 nm wavelengths. Total soluble sugar was determined on dry samples using the phenol sulfuric acid method (Dubois et al., 1956).

Data analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS v. 25.0). A two-way analysis of variance was used to determine whether species, season, and speciesXseason affected Ψ_{TLP}, Ψ_{100}, Ɛmax, V0/DW, RWC, soluble sugar, and photosynthetic pigment. Duncan's test was used to compare the means. The student's t-test was used to determine any significant difference in physiological and biochemical properties between summer and winter. Principal component analysis (PCA) was also used to determine the relationship between variables and physiological and biochemical properties.

RESULTS

Osmotic potential at turgor loss point depended is seasonal and species dependent, while the osmotic potential at full turgor is species dependent (Table 2; P<0.05). *J. excelsa* showed higher osmotic potential at turgor loss point in summer than in winter. In summer, *J. foetidissima* showed lower osmotic potential at the turgor loss point and osmotic potential at full turgor than *J. excelsa* and *J. oxycedrus*. There was no significant difference in osmotic potential at full turgor between the species in winter (Figure 2).

Table 2. The statistical significance (P>F) of main effects of season, species and seasonxspecies interactions on Ψ_{TLP}, Ψ_{100}, Ɛmax, V0/DW, RWC, TSS, Chla, Chlb and Chla+b.

Factors	Season	Species	Season x Species
Ψ_{TLP} (MPa)	**<0.01**	**<0.001**	0.177
Ψ_{100} (MPa)	0.889	**<0.05**	0.378
Ɛmax (MPa)	0.883	**<0.01**	0.443
V0/DW	0.335	0.496	0.632
RWC (%)	**<0.001**	**<0.01**	0.129
TSS (mg g^{-1} DW)	**<0.001**	**<0.001**	0.077
Chla (mg g^{-1})	**<0.001**	**<0.001**	**<0.05**
Chlb (mg g^{-1})	**<0.001**	0.588	0.129
Chla+b (mg g^{-1})	**<0.001**	**<0.01**	**<0.05**

Significant effects are highlighted in bold.

Season and species affected RWC and species affected Ɛmax (Table 2; P<0.05). According to the pressure-volume (P-V) curve analysis, *J. foetidissima* showed highest Ɛmax in summer (Fig. 3a). *J. excelsa* and *J. foetidissima* had higher RWC than *J. oxycedrus* in winter (Fig. 3c).

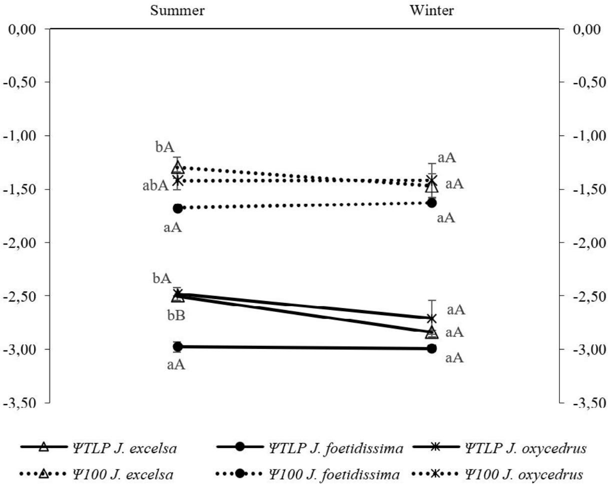

Figure 2. Changes in Ψ_{TLP} (osmotic potential at turgor loss point) and Ψ_{100} (osmotic potential at full turgor) in summer (2016 year) and winter (2017 year) seasons in *Juniperus excelsa*, *Juniperus foetidissima* and *Juniperus oxycedrus* (Means ± standard error; capital letters mean difference between in seasons; lower case letters mean the difference between species.

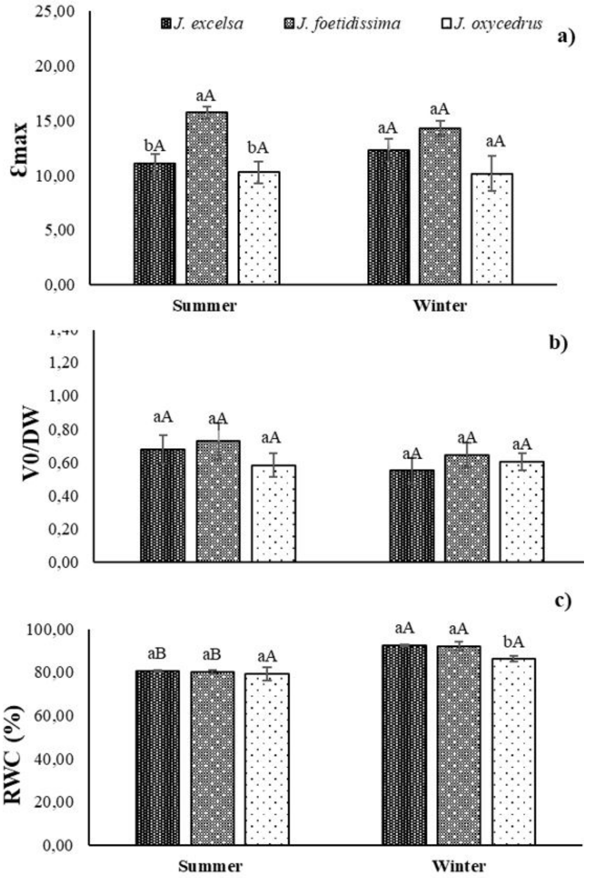

Figure 3. Plant water relation parameters [a:Ɛmax (MPa), b:V0/DW, c:RWC (%)] in summer (2016 year) and winter (2017 year) seasons in *Juniperus excelsa*, *Juniperus foetidissima* and *Juniperus oxycedrus* (Means ± standard error; capital letters mean difference between in seasons; lower case letters mean the difference between species.

Season and species affected total soluble sugar, Chla, and Chla+b. The total soluble sugar content, which was high in spring, decreased in summer but increased again in autumn and winter in all species (Fig. 4). All three species had the lowest soluble sugar content in summer. *J. oxycedrus* showed highest total soluble sugar content in summer and winter.

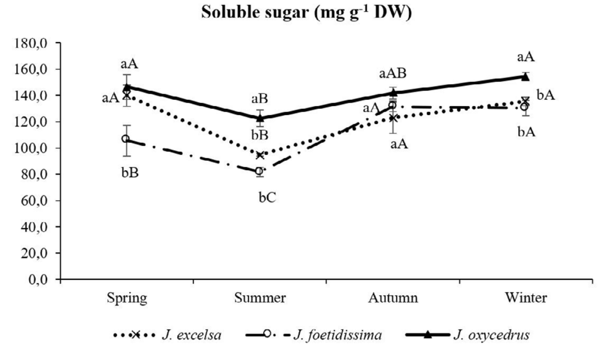

Figure 4. Variation of total soluble sugar content according to seasons (2016-2017 years) in *Juniperus excelsa*, *Juniperus foetidissima* and *Juniperus oxycedrus* (Means ± standard error; capital letters mean difference between in seasons; lower case letters mean the difference between species.

Photosynthetic pigment content, which was generally low in spring, increased in summer and then decreased towards autumn and winter. *J. oxycedrus* showed highest and J.excelsa showed lowest Chla in the spring, autumn and winter. All three species had similar photosynthetic pigment content in summer (Fig. 5a-b-c).

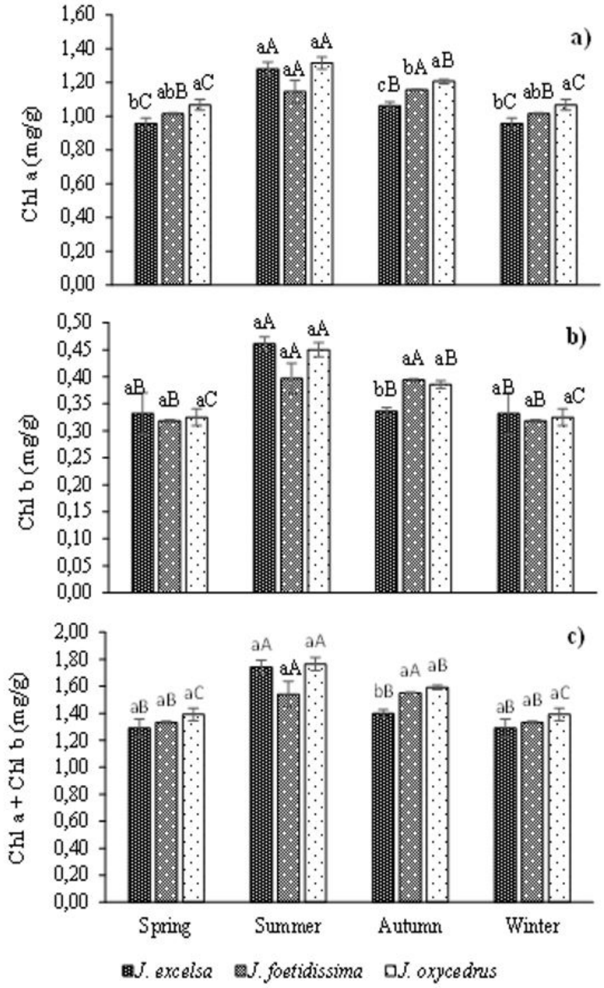

Figure 5. Variation of photosynthetic pigment content (a:Chl a, b:Chl b and c:Chla+Chlb) according to seasons (2016-2017 years) in *Juniperus excelsa*, *Juniperus foetidissima* and *Juniperus oxycedrus* (Means ± standard error; capital letters mean difference between in seasons; lower case letters mean the difference between species.

According to the PCA analysis, RWC (r:0.854), TSS (r:0.621), Chla (r:-0.918), Chlb (r:-0.883), Total Chl (r:-0.933), mean air temperature (r:-0.921), and total precipitation (r:0.921) had the highest correlation with Axis 1 (52.8 %) (Fig. 6). According to Axis 1, there was a correlation between air temperature and Chl a, Chl b, and total chlorophyll content, while there was a correlation between total precipitation and RWC and TSS. According to the PCA analysis, Ψ_{TLP} (r:-0.615), Ψ_{100} (r:-0.669) and Ɛmax (r:0.791) had the highest correlation with Axis 2 (25.5%). There was a correlation between osmotic potential at the turgor loss point, osmotic potential at full turgor and Emax.

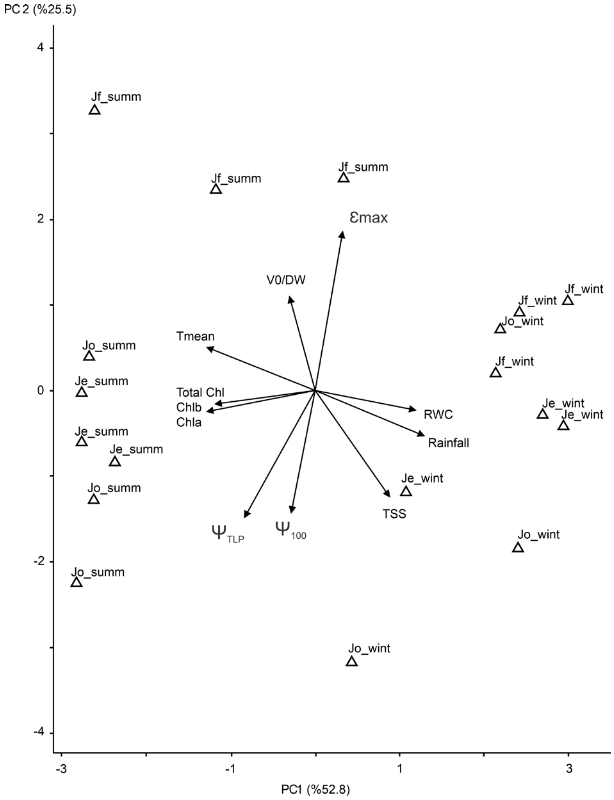

Figure 6. Principal component analysis (PCA) of Ψ_{TLP}, Ψ_{100}, Ɛmax , V0/DW, RWC, TCC, Chla, Chl b and total Chl in *J. foetidissima* (*Jf*), *J. excelsa* (*Je*) and *J. oxycedrus* (*Jo*) (wint: winter, summ:summer).

DISCUSSION

Ψ_{TLP} measurements showed that *J. excelsa* and *J. oxycedrus* reach their Ψ_{TLP} earlier than *J. foetidissima* under water limitations in summer, while all three species had similar Ψ_{TLP} in winter. Research shows a reduction in Ψ_{TLP} in response to cold and drought depending on the species (Anisko and Lindstrom, 1996; White et al., 1996; Maréchaux et al., 2015). *J. foetidissima* had the lowest Ψ_{TLP} in the summer and improved drought tolerance compared to the other two species. The species with the least negative osmotic potential at turgor loss point is least adapted to drought (Duhme and Hinckley, 1992). Plants with more negative Ψ_{TLP} can resist leaf dehydration and thus maintain stomatal conductance, photosynthesis, and growth under lower water availability (Tognetti et al., 2000). *J. foetidissima* showed highest Ɛmax in summer. The lower Ψ_{TLP} and Ψ_{100}, the higher Ɛmax (i.e.the lowest tissue elasticity). In summer, *J. foetidissima* showed high Ɛmax, retaining more symplastic water within its cells during the reduction of turgor potential. Cells with a high elastic modulus retain more water than other cells at or near Ψ_{TLP} (Colombo, 1987). With low cell wall elasticity, *J. foetidissima* showed higher tolerance and adaptation than other *Juniperus* species in water deficit. Plants with high Ɛmax are more drought tolerant (Ritchie and Schula, 1984). Ψ_{TLP} varies from season to season (Mitchell et al., 2008; Deligöz et al., 2021). Differences in physiological responses to water deficiency show that alternative mechanisms (osmotic or elastic adjustment) are important for leaf tissue integrity and survival (Mitchell et al., 2008). The osmotic and elastic adaptability of woody plants varies from species to species (Sanders and Arndt, 2012). It is related to plant phenology (Kubiske and Abrams, 1991) and is largely dependent on environmental conditions (Leuschner et al., 2019). In the present study, mean air temperature and total precipitation were related to total soluble sugar and photosynthetic pigment contents. *J. excelsa* had lower Ψ_{TLP} but a higher total soluble sugar content in the winter than in the summer. In general, the low osmotic potential in winter may be related to an increase in the number of osmolytes dissolved in leaf cells. In addition, this decrease may be a cold hardening reaction (Harayama et al., 2006). Ritchie and Schula (1984) reported that the decrease in the osmotic potential at the turgor loss point and full turgor in *Pseudotsuga menzeisii* (Mirb.) might be due to sugar accumulation. On the other hand, Gross and Koch (1991) stated that the decrease in the osmotic potential at the turgor loss point and full turgor in *Picea abies* L. might be due to the change in symplastic volume rather than the change in soluble content. All three species had similar V0/DW in the summer and winter. In general, symplastic water at a saturated point per dry weight of the shoot is high in spring but decreases in summer and winter (Tognetti et al., 2000). Relative water content is a sensitive variable that responds rapidly to environmental conditions (temperature, light, humidity, and water supply) (Tanentzap, 2015) and directly reflects the water status of plants (Yang and Miao, 2010). Compare to the species, *J. excelsa* and *J. foetidissima* showed higher RWC than *Juniperus oxycedrus* in winter. A relative water content of 40 to 60% results in desiccation damage (Vostral et al., 2002). None of the species had an RWC of lower than 86%. High leaf water content converts excess energy into heat and reduces damage to chloroplasts (Tomlinson et al., 2013).

Total precipitation was correlated with RWC and TSS. Generally, non-structural carbohydrates are high in winter, decrease in summer, and increase again (Diamantoglou et al., 1989; Palacio et al., 2018). During cold months, soluble sugar content increases as starch concentrations decrease. This sugar may play a role in cold tolerance (Wong et al., 2003). In the summer and winter, *J. oxycedrus* had higher total soluble sugar content than *J. excelsa* and *J. foetidissima*. However, all three species had similar total soluble sugar content in the autumn. High sugar content in autumn may make plants more resistant to cold (Schaberg et al., 2000). In autumn and winter, soluble carbohydrates cause an increase in leaf concentration, which has been associated with frost hardening (Oleksyn et al., 2000). The number of frost days in the experimental plot was 22 in January. Soluble sugar content increased in the autumn and winter compared to the summer. This may also be related to cold tolerance. Woody plants can adapt to temperatures below freezing point to survive cold stress. First, they get used to it partially in short days. Later, low temperatures and prolonged subfreezing temperatures trigger the plant, resulting in midwinter cold hardiness (Weiser, 1970). Trees also need to store reserves to survive winter and burst buds and grow shoots the following spring (Regier et al., 2010). Seasonal changes in carbohydrate concentration can be largely explained by leaf phenology (Neweel et al., 2002). Photosynthetic pigment content is another parameter that changes seasonally. Photosynthetic pigment content, which was generally low in the spring and winter, was high in the summer. Plants adjust chlorophyll content to adapt to environmental conditions (Bayar and Deligöz, 2021). Photosynthetic pigments usually increase at the end of the growing season. The high level of chlorophyll pigments late in the season (august) is an adaptive protective mechanism in stressed plants (Kulaç et al., 2012). There was a positive correlation between annual mean maximum temperature and photosynthetic pigment content (Yücedağ et al., 2021). All three species had higher Chla, Chlb, and Chla+b with an increase in average air temperature. Season affected photosynthetic pigment content (Wolkerstorfer et al., 2011; Bündchen et al., 2016). Seasonal changes in photosynthetic pigment content (Chla+b) are controlled by the daily light period. The best frost-resistant needles contain fewer photosynthetic units (Vogg et al., 1998). There is a consistent relationship between photosynthetic pigment content and light (Minotta and Pinzauti 1996) and temperature affects photosynthetic pigment content (Ottander et al., 1995). *J. excelsa* showed lowest Chla in the winter. Photosynthetic pigment content varies by year, season, and species (Uvalle Sauceda et al., 2008).

CONCLUSION

Ecophysiological processes are the basis of evolutionary adaptation to climate change (Chmura et al., 2011). Reductions in elasticity (high εmax) were associated with drought tolerance. *Juniperus* species had different elastic responses to drought. Results showed that *J. foetidissima* is more drought tolerant and adapted to summer water deficit better than *J. excelsa* and *J. oxycedrus*. Ψ_{TLP} provides important information about a species' capacity to tolerate drought conditions and can be used to select drought-tolerant species. The cold tolerance of the species (similar Ψ_{TLP}, Ψ_{100}, and εmax) in winter was also similar. We should make more measurements detailing the selection criteria for cold resistance and conduct more research on the role of water relations parameters (Ψ_{TLP}, Ψ_{100}, and εmax) and soluble carbohydrate content in osmotic regulation in seasonal variation.

AUTHORSHIP CONTRIBUTION

Project Idea: AD
Database: AD, EB
Processing: AD, EB
Analysis: EB
Writing: AD, EB
Review: AD, EB

REFERENCES

ANISKO, T.; LINDSTROM, O.M. Cold hardiness and water relation parameters in *Rhododendron* cv. Catawbiense Boursault subjected to drought episodes. Physiologia Plantarum, v.98, p.147-155, 1996.

ANŞİN, R.; ÖZKAN, Z.C. Tohumlu Bitkiler (*Spermatophytha*) Odunsu Taksonlar. Karadeniz Teknik Üniversitesi Basımevi, Trabzon. 2006.450p.

ARNON, D.I. Copper enziymes in izolated chloroplasts polyphenoioxidase in *Beta vulgaris*. Plant Physiolgy, v. 24, p.1-15, 1949.

BACELAR, E.A.; MOUTINHO-PEREIRA, J.M.; GONCALVES, B.C.; LOPES, J.I.; CORREIA, C.M. Physiological responses of different olive genotypes to drought conditions. Acta Physiol Plant, v.31, p.611-621, 2009.

BARTLETT, M.K., SCOFFONI, C.; ARDY, R., ZHANG, Y.; SUN, S.; CAO, K.; SACK, L. Rapid determination of comparative drought tolerance traits: using an osmometer to predict turgor loss point. Methods in Ecology and Evolution, v.3, p.880-888, 2012.

BARTLETT, M.K.; KLEIN, T.; JANSEN, S.; CHOAT, B.; SACK, L. The correlations and squence of plant stomatal, hydraulic, and wilting responses to drought. PNAS, v.113, n.46, p.13098-13103, 2016.

BAYAR, E.; DELIGOZ, A. Ecophysiological behavior of Mediterranean woody species under summer drought. Bosque, v42, n.3, p.311-321, 2021.

BUNDCHEN, M.; BOEGER, MRT.; REISSMANN, C.B.; GERONAZZO, K.M. Interspecific variation in leaf pigments and nutrients of five tree species from a subtropical forest in southern Brazil. Annals of the Brazilian Academy of Sciences, v. 88, n. 1, p.467-477, 2016.

CHARRA-VASKOU, K.; CHARRIER, G.; WORTEMANN, R.; BEIKIRCHER, B.; COCHARD, H.; AMEGLIO, T.; MAYR, S. Drought and frost resistance of trees: a comparison of four species at different sites and altitudes. Annals of Forest Science, v.69, p.325-333, 2012.

CHARRIER, G.; NGAO, J.; SAUDREAU, M.; AMÉGLIO, T. Effects of environmental factors and management practices on microclimate, winter physiology, and frost resistance in trees. Frontiers in Plant Science, v. 6, n.259, p.1-18, 2015.

CHMURA, D.J.; ANDERSON, P.D.; HOWE, G.T.; HARRINGTON, C.A.; HALOFSKY, J.E.; PETERSON, D.L.; SHAW, D.C.; ST. CLAIR, J.B. Forest responses to climate change in the Northwestern United States: Ecophysiological foundations for adaptive management. Forest Ecology and Management, v.261, p.1121-1142, 2011.

COLOMBO, S.J. Changes in osmotic potential, cell elasticity and turgor relationships of 2nd year black spruce container seedlings. Can. J. For. Res. v.17, p.365-369, 1987.

COLOMBO, S.J.; TENG, Y. Seasonal variation in the tissue water relations of *Picea glauca*. Oecologia, v.92, p.410-415, 1992.

CORREIA, M.J.; COELHO, D.; DAVID, M.M. Response to seasonal drought in three cultivars of *Ceratonia siliqua*:leaf growth and water relations. Tree Physiology, v.21, p.645-653, 2001.

DELİGÖZ, A.; BAYAR, E.; GENÇ, M.; KARATEPE, Y. Differences in water relations and total carbohydrate content between precommercially thinned and unthinned *Pinus nigra* subsp. *pallasiana* stands. Journal of Forest Science, v.67, n.3, p.125-133, 2021.

DIAMANTOGLOU, S.; RHIZOPOULOU, S.; HERBIG, A.; KULL, U. Seasonal trends in energy content and storage substances in mediterranean shrub *Ephedra*. Acta Ecologica, v.10, n.3, p.263-274, 1989.

DİRİK, H. Üç yerli çam türünün (*Pinus brutia* Ten., *Pinus nigra* Arn. ssp. *pallasiana* Lamb. Holmboe, *Pinus pinea* L.) kurak peryottaki transpirasyon tutumlarının ekofizyolojik analizi. İstanbul Üniversitesi Orman Fakültesi Dergisi, v.44, n.1, p.111-121,1994.

DOUAIHY, B.; RESTOUX, G.; MACHON, N.; DAGHER-KHARRAT, M.B. Ecological characterizationof the *Juniperus excelsa* stands in Lebanon. Ecologia Mediterranea, v.39, n.1, p.169-180, 2013.

DUBOIS, M.; GILLES, K.; HAMILTON, J.K.; REBERS, P.A.; SMITH, F. Calorimetric method for determination of sugars and related substances. Analytical Chemistry, v.28, p. 350–356, 1956.

DUHME, F.; HINCKLEY, T.M. Daily and seasonal variation in water relations of macchia shrubs and trees in France (Montpellier) and Turkey (Antalya). Vegetatio, v.99/100, p.185-198, 1992.

ELER, Ü. Ardıç Ormanlarımız. Süleyman Demirel Üniversitesi Orman Fakültesi Dergisi, v.A1, p.87-96, 2000.

GENC, M.; YAHYAOGLU, Z. Kalite sınıflamasında kullanılan özellikler ve tespiti. In: YAHYAOGLU, Z.; GENC, M. Fidan Standardizasyonu Standart Fidan Yetiştirmenin Biyolojik ve Teknik Esasları. Suleyman Demirel University, Isparta. 2007. p. 355-465.

GROSS, K.; KOCH, W. Water relations of *Picea abies* I. Comparison of water relations parameters of spruce shoots examines at the end of the vegetation period and in winter. Physiologia Plantarum, v.83, p.290-295, 1991.

GÜLTEKİN, H.C.; GÜLCÜ, S.; GÜLTEKİN, Ü.G.; DİVRİK, A. Studies on determination the effects of some practicable classification methods on seed germination of Crimean Juniper (*Juniperus excelsa* Bieb.) before sowing process. Kafkas University Artvin Journal of Forestry Faculty, v.1 n. 2, p.111-120, 2003.

HARAYAMA, H.; IKEDA, T.; ISHIDA, A.; YAMAMOTO, S. Seasonal variations in water relations in current-year leaves of evergreen trees with delayed greening. Tree Physiology, v.26 p.1025-1033, 2006.

İMAL, B. Ecophysiological determination of cold and drought tolerances of some Anatolian black pine (*Pinus nigra* Arnold ssp. *pallasiana* [Lamb.] Holmboe) origins. 2015. 164p. PhD Thesis İstanbul University, İstanbul.

KUBISKE, M.E.; ABRAMS, M.D. Seasonal, diurnal and rehydration-induced variation of pressure-volume relationships in *Pseudotsuga menziesii*. Physiologia Plantarum v.83, p.107-116, 1991.

KULAÇ, Ş.; NZOKOU, P.; GÜNEY, D.; CREGG, B.M.; TURNA, İ. Growth and physiological response of fraser fir [*Abies fraseri* (Pursh) Poir.] seedlings to water stress: seasonal and diurnal variations in photosynthetic pigments and carbohydrate concentration. American Society for Horticultural Science, v.47, n.10, p.8, 2012

KUNERT, N. Preliminary indications for diverging heat and drought sensitivities in Norway spruce and Scots pine in central Europe. iForest Biogeosciences and Forestry, v.13 p.89-91, 2020.

KUNERT, N.; ZAILAA, J.; HERRMANN, V.; MULLER-LANDAU, H.C.; WRIGHT, S.C.; PÈREZ, R.; McMAHON, S.M.; CONDIT, R.C.; HUBBELL, S.P.; SACK, L.; DAVIES, S.T.; ANDERSON-TEIXEIRA, K.J. Leaf turgor loss point shapes and regional distributions of evergreen but not deciduod tropical trees. New Phytologist, v.230, p.485-496, 2021.

LAMBERS, H.; CHAPIN III, F.S.; PONS, T.L. Plant Physiological Ecology. 2nd ed. Springer, 2008.540p.

LEUSCHNER, C.; WEDDE, P.; LUBBE, T. The relation between pressure-volume curve traits and stomatal regulation of water potential in five temperate broadleaf species. Annals of Forest Science, v.76, n.60, 2019.

LEVITT, J. Responses of plants to environmental stresses. 2nd ed. Academic Press, NewYork.1980. 497p.

MARÉCHAUX, I.; BARTLETT, M.K.; SACK, L.; BARALOTO, C.; ENGEL, J.; JOETZJER, E.; CHAVE, J. Drought tolerance as predicted by leaf water potential at turgor loss point varies strongly across species within an Amazonian forest. Functional Ecology, v.29, p.1268-1277, 2015.

MICHELOZZI, M.; JOHNSON, J.D.; WARRAG, E.I. Response of ethlene and chlorophyll in two eucalyptus clones during drought. New Forests, v.9 p.197-204, 1995.

MINOTTA, G.; PINZAUTI, S. Effects of light and soil fertility on growth, leaf chlorophyll content and nutrient use efficiency of beech (*Fagus sylvatica* L.) seedlings. Forest Ecology and Management, v.86, p. 61-71, 1996.

MITCHELL, P.J.; VENEKLAAS, E.J.; LAMBERS, H.; BURGESS, S.S.O. Leaf water relations during summer water deficit: differential responses in turgor maintenance and variation in leaf structure among different plant communities in south-western Australia. Plant, Cell and Environment, v.31, n. 1791-1802, 2008.

NEWEEL, E.A.; MULKEY, S.S.; WRIGHT, S.J. Seasonal patterns of carbohydrate storage in four tropical tree species. Oecologia, v.131, p.333-342, 2002.

OGM (FOREST GENERAL DIRECTORATE). Ormancılık İstatistikleri 2020. Available et:https://www.ogm.gov.tr/tr/e-kutuphane/resmi-istatistikler. Accessed in:April 10th 2021.

OLEKSYN, J.; ZYTKOWIAK, R.; KAROLEWSKI, P.; REICH, P.B.; TJOELKER, M.G. Genetic and environmental control of seasonal carbohydrate dynamics in tress of diverse *Pinus sylvestris* populations. Tree Physiology, v.20, p. 837-847, 2000.

OLIVERIA, G.; CORREIA, O.A.; MARTINS-LAUÇÃO, M.A.; CATARINO, F.M. Water relations of cork-oak (*Quercus suber* L.) under natural conditions. Vegetatio, v.99-100, p.199-208, 1992.

OTTANDER, C.; CAMPBELL, D.; OQUIST, G. Seasonal changes in photosystem II organisation and pigment composition in *Pinus sylvestris*. Planta, v.197, p.176-183, 1995.

OUYANG, L.; LEUS, L.; DE KEYSER, E.; VAN LABEKE, M.C. Seasonal changes in cold hardiness and carbohydrate metabolism in four garden rose cultivars. Journal of Plant Physiology, v.232, p.188-199, 2019.

PALACIO, S.; CAMARERO, J.J.; MAESTRO, M.; ALLA, A.Q.; LAHOZ, E.; MONTSERRAT-MARTÍ, G. Are storage and tree growth related? Seasonal nutrient and carbohydrate dynamics in evergreen and deciduous Mediterranean oaks. Trees, v.32, p.777-790, 2018.

PARKER, W.C.; PALLARDY, S.G. Pressure-volume analysis of leaves of *Robinia pseudoacacia* L. with the sap expression and free transpiration methods. Can J. For. Res., v.18, p.1211-1213, 1988.

PARKER, W.C.; PALLARDY, S.G.; HINCKLEY, T.M.; TESKEY, R.O. Seasonal changes in tissue water relations of three woody species of the *Quercus carya* forest type. Ecology, v.63, n.5, p.1259-1267, 1982.

PITA, P.; PARDOS, J.A. Growth, leaf morphology, water use and tissue water relations of Eucalyptu*s globus* clones in response to water deficit. Tree Physiology, v.21, p.599-607, 2001.

REGIER, N.; STREB, S.; ZEEMAN, S.C.; FREY, B. Seasonal changes in starch and sugar content of poplar (*Populus deltoides* x *nigra* cv. Dorskamp) and the impact of stem girdling on carbohydrate allocation to roots. Tree Physiology, v.30, p.979-987, 2010.

RITCHIE, G.A. Assessing seedling quality. In: DURYEA, M.L.; LANDIS, T.D. Forest nursery manual production of bareroot seedlings. 1984. p.243-259.

RITCHIE, G.A.; SHULA, R.G. Seasonal changes of tissue-water relations in shoots and root systems of Douglas-fir seedlings. Forest Science, v.30, n.2, p.538-548, 1984.

SAITO, T.; SOGA, K.; HOSON, T.; TERASHIMA, I. The bulk elastic modulus and the reversible properties of cell walls in developing *Quercus* leaves. Plant Cell Physiol., v.47, n.6, p.715-725, 2006.

SANDERS, G.J.; ARNDT, S.K. Osmotic adjustment under drought conditions. In: AROCA, R. Plant responses to drought stress from morphological to molecular features. 2012. p 199–229.

SCHABERG, P.G.; SNYDER, M.C.; SHANE, J.B.; DONNELLY, J.R. Seasonal patterns of carbohydrate reserves in red spruce seedlings. Tree Physiology, v.20, pp.549-555, 2000.

SCHOLANDER, P.F.; HAMMEL, H.T.; BRADSTREET, E.D.; HEMMİNGSEN, E.A. Sap pressure in vascular plants. Science, v.148, p.339- 346, 1965.

SCHOLZ, F.G.; BUCCI, S.J.; ARIAS, N.; MEINZER, F.C.; GOLDSTEIN, G. Osmotic and elastic adjustments in cold desert shrubs differing in rooting depth: coping with drought and subzero temperatures. Oecologia, v.170, p. 885-897, 2012.

SÈNE, E-H. M. Silviculture and management in arid and semi-arid regions. Food And Agriculture Organization, Elsevier, 2004.

TANENTZAP, F.M.; STEMPEL, A.; RYSER, P. Reliability of leaf relative water content (RWC) measurements after storage: consequences for in situ measurements. Botany, v.93 p.1-7, 2015.

TOGNETTI, R.; RASCHI, A.; JONES, M.B. Seasonal patterns of tissue water relations in three Mediterranean shrubs co-occuring at a natural CO_2 spring. Plant, Cell and Environment, v.23 p.1341-1351, 2000.

TOMLINSON, K.W.; POORTER, L.; STERCK, F.C.; BORGHETTI, F.; WARD, D.; DE BIE, S.; VAN LANGEVELDE, F. Leaf adaptations of evergreen and deciduous trees of semi-arid and humid savannas on three continents. Journal of Ecology, v.101, p.430-440, 2013.

TYREE, M.T.; RICHTER, H. Alternate methods of analysing water potential isotherms: some cautions and clarifications. II. Curvilinearity in water potentia isotherms. Can. J. Bot., v.60, p.911-916, 1982.

UVALLE SAUCEDA, J.I.; GONZÁLEZ RODRÍGUEZ, H.; RAMÍREZ LOZANO, R.G.; CANTÚ SILVA, I.; GÓMEZ MEZA, M.V. Seasonal trends of chlorophylls a and b carotenoids in native trees and shrubs of northeastern Mexico. Journal of Biological Sciences, v.8 n.2, p.258- 267, 2008.

VILLAR, E.; KLOPP, C.; NOIROT, C.; NOVAES, E.; KIRST, M.; PLOMION, C.; GION, J.M. RNA-Seq reveals genotype-specific molecular responses to water deficit in eucalyptus. BMC Genomics, v.12, n.538, p.1-18, 2011.

VOGG, G.; HEIM, R.; HANSEN, J.; SCHÄFER, C.; BECK, E.. Frost hardening and photosynthetic performance of Scots pine (*Pinus sylvestris* L.) needles. I. Seasonal changes in the photosynthetic apparatus and its function. Planta, v.204, p.193-200, 1988.

VOSTRAL, C.B.; BOYCE, R.L.; FRIEDLAND, A.J. Winter water relations of New England conifers and factors influencing their upper elevational limits. I. Measurements. Tree Physiology, v.22, p.793-800, 2002.

WEISER, C.F. Cold resistance and injury in woody plants. Science. V.169, p.1269-1278,1970.

WHITE, D.A.; BEADLE, C.L.; WORLEDGE, D. Leaf water relations of *Eucalyptus globulus* ssp. globulus and *E. nitens*: seasonal, drought and species effects. Tree Physiology, v.16, p.469-476, 1996.

WOLKERSTORFER, S.V.; WONISCH, A.; STANKOVA, T.; TSVETKOVA, N.; TAUSZ, M. Seasonal variations of gas exchange, photosynthetic pigments, and antioxidants in Turkey oak (*Quercus cerris* L.) and Hungarian oak (*Quercus frainetto* Ten.) of different age. Trees, v.25, p.1043-1052, 2011.

WONG, B.L.; BAGGETT, K.L.; RYE, A.H. Seasonal patterns of reserve and soluble carbohydrates in mature sugar maple (*Acer saccharum*). Can. J. Bot., v.81, p.780-788, 2003.

YANG, F.; MIAO, L.F. Adaptive responses to progressive drought stress in two poplar species originating from different altitudes. Silva Fennica, v.44, n.1, pp.23-37, 2010.

YÜCEDAĞ, C.; AYAN, S.; FARHAT, P.; ÖZEL, H.B. *Juniperus* L. for restoration of degraded forest lands in Turkey. Seefor, v.12, n.1, p.71-81, 2021.

ZHU, S.D.; CHEN, Y.L.; YE, Q.; HE, P.C.; LIU, H.; LI, R.H.; FU, P.L.; JIANG, G.F.; CAO, K.F. Leaf turgor loss point is correlated with drought tolerance and leaf carbon economics traits. Tree Physiology, v.38, p.658-663, 2018.