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Research article (Araştırma makalesi)

Impact of mistletoe infestation on tree water status in Anatolian black pine trees in a semi-arid Mediterranean forest stand

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Abstract: Mistletoes can adversely affect the hydraulic functioning of their host trees by continuously extracting water and nutrients. This study investigated the effects of pine mistletoe (*Viscum album* subsp. *austriacum*) on water status in Anatolian black pine (*Pinus nigra* subsp. *pallasiana*) trees in a natural forest stand in Mediterranean Region, Türkiye, where mistletoe-infected and non-infected trees coexisted. Continuous stem radius change measurements were obtained throughout the 2024 growing period using automatic band dendrometers to compare maximum daily shrinkage (MDS) and tree water deficit (TWD) between infected and non-infected trees. Simultaneously, soil water potential was measured, and meteorological variables were recorded by a nearby station. Mistletoe-infected trees exhibited 92% higher TWD and 12% higher MDS compared to non-infected trees. In both groups, TWD and MDS were closely associated with meteorological variables. TWD showed significant correlations with soil water potential, global radiation, and vapor pressure deficit, while MDS was mainly related to air temperature, solar radiation, and soil water potential. Precipitation influenced MDS in non-infected trees but had no significant effect on infected trees. Moreover, correlation coefficients with environmental factors were consistently higher in infected trees. These findings indicate that pine mistletoe is associated with a marked deterioration in host water status, suggesting increased drought sensitivity and reduced physiological resilience under Mediterranean climatic conditions. Overall, this highlights the potential ecological risk posed by mistletoe infestation in drought-prone forest ecosystems.
Keywords: Drought stress, Maximum daily shrinkage, *Pinus nigra*, Tree water deficit, *Viscum album* parasitism

Yarı kurak bir Akdeniz orman ekosisteminde ökse otunun karaçam ağaçlarının su durumu üzerindeki etkisi

Öz: Ökse otları, konukçu ağaçtan sürekli su ve mineral madde alarak konukçu ağacın hidrolik işlevini olumsuz etkileyebilmektedir. Bu çalışma, çam ökse otunun (*Viscum album* subsp. *austriacum*) karaçam (*Pinus nigra* subsp. *pallasiana*) ağaçlarının su durumu üzerindeki etkilerini araştırmayı amaçlamaktadır. Araştırma, Türkiye'nin Akdeniz Bölgesi'nde, hem ökse otu ile enfekte olmuş hem de sağlıklı karaçam bireylerinin bir arada bulunduğu doğal bir meşcerede yürütülmüştür. 2024 yılı büyüme dönemi boyunca, enfekte ve sağlıklı ağaçlara kurulan otomatik şerit dendrometreler ile gövde yarıçapı değişiklikleri sürekli olarak kaydedilmiş; bu veriler kullanılarak maksimum günlük daralma (MGD) ve ağaç su açığı (ASA) parametreleri hesaplanmıştır. Eşzamanlı olarak toprak su potansiyeli ölçülmüş ve sahaya kurulan meteoroloji istasyonu aracılığıyla meteorolojik değişkenler izlenmiştir. Sonuçlar, enfekte ağaçların sağlıklı ağaçlara kıyasla %92 daha yüksek ASA ve %12 daha yüksek MGD değerlerine sahip olduğunu göstermiştir. Hem enfekte hem de sağlıklı ağaçlardaki ASA ve MGD değerlerinin meteorolojik değişkenlerle yakından ilişkili olduğu belirlenmiştir. ASA, toprak su potansiyeli, küresel radyasyon ve buhar basıncı açığı ile güçlü korelasyon gösterirken, MGD hava sıcaklığı, güneş radyasyonu ve toprak su potansiyeli ile ilişkili bulunmuştur. Yağış, sağlıklı ağaçların MGD'si üzerinde etkili olurken, enfekte ağaçlar üzerinde anlamlı bir etkisi gözlenmemiştir. Ayrıca, ağaç su durumu parametreleri ile çevresel faktörler arasındaki korelasyon katsayılarının enfekte ağaçlarda daha yüksek olduğu tespit edilmiştir. Bulgular, ökse otunun karaçam ağaçlarında su durumunu olumsuz etkilediğini, bu durumun Akdeniz iklim koşullarında enfekte ağaçların kuraklığa duyarlılığını artırabileceğini ve fizyolojik dayanıklılığını azaltabileceğini ortaya koymaktadır.
Anahtar kelimeler: Kuraklık stresi, Maksimum günlük daralma, *Pinus nigra,* Ağaç su açığı, *Viscum album* parazitizmi

1. Introduction

The Mediterranean region is highly vulnerable to climate change, with projected increases in temperature (0.9–5.6 °C) and decreases in precipitation (4–22%) by the end of the 21st century (Ali et al. 2022). Temperature anomalies and drought events driven by climate change pose substantial threats to the health of most vegetation types in the region, particularly to forest ecosystems (Hidalgo-Triana et al., 2023). Future climate projections based on IPCC scenarios (SRES, RCP, SSP) indicate that shifts in temperature and precipitation regimes will challenge the adaptive capacity of Mediterranean forest species, increasing their vulnerability to both abiotic and biotic stressors (Özdemir et al., 2020). Climate-induced alterations in forest structure and function can undermine critical ecosystem services such as water regulation, erosion control, and carbon sequestration, thereby reducing the functioning of Mediterranean watersheds (Özkan et al., 2021). Reports indicate mass tree mortality in various tree species due to increasing drought effects in the

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Mediterranean region (Gentilesca et al., 2017; Senf et al., 2020; Vatandaşlar et al., 2023; Alderotti et al., 2024). This threat is further exacerbated by biotic stresses, such as parasitic infections, which cause considerable ecological and economic damage to forests (Teshome et al., 2020).

Mistletoes, including *Viscum album* and other species within the *Viscum* genus, are semi-parasitic flowering plants that extract water and dissolved mineral nutrients from their host trees through haustoria, specialized structures that tap into the host xylem (Muche et al., 2022). Infested trees experience nutrient depletion, reduced carbohydrate reserves, impaired growth, morphological abnormalities, and increased susceptibility to pests and diseases (Zuber, 2004; Szmidla et al., 2019).

Globally, mistletoes include approximately 100 species (Nadkarni et al., 2001; Szurpnicka et al., 2020), but in Türkiye, only three species are found: *Viscum album* L., *Loranthus europaeus* Jacq., and *Arceuthobium oxycedri* (DC.) M. Bieb. (Üstüner, 2018). Among these, *V. album* is the most widespread, with three subspecies affecting different tree groups: (i) *V. album* subsp. *album* L. (broadleaf hosts), (ii) *V. album* subsp. *austriacum* (Wiesb.) Vollmann (pine and spruce), and (iii) *V. album* subsp. *abietis* (Wiesb.) Abromeit (fir). In Türkiye, mistletoe infestation is particularly problematic in coniferous forests, severely impacting Anatolian black pine (*Pinus nigra*), Scots pine (*P. sylvestris*) and fir (*Abies* spp.) (Yüksel et al., 2005; Sakıcı et al., 2022; Sakıcı et al., 2023).

Previous research has highlighted a wide range of mistletoe-related effects on forest trees in Türkiye; including reduced radial growth in Scots pine (Sönmez, 2014; Bilgili et al., 2018), in Anatolian black pine (Kanat et al., 2010; Çatal and Carus, 2011; Sakıcı et al., 2023) and in fir (Sakıcı et al., 2022; Öztürk, 2023). Other works have reported infection rates (Usta and Yılmaz, 2021; Baysal, 2023), host-species preferences (Kumbaşlı et al., 2011), shifts in foliar chemistry (Mutlu et al., 2016), changes in leaf morphology and anatomy (Öztürk et al., 2022), reductions in needle size (Bilgili et al., 2020); modifications of wood anatomy (Göl et al., 2018), and a reduced capacity of sapwood to support foliage in infected hosts (Bilgili et al., 2020). Yet the hydrophysiological consequences of mistletoe infection, particularly its influence on tree water status, remain poorly documented, especially in Mediterranean forests where drought frequency is rising and regional temperatures are increasing about 20 % faster than the global average (Lionello and Scaracia, 2018).

This study aimed to assess the impact of pine mistletoe (*Viscum album* subsp. *austriacum* (Wiesb.) Vollmann) infestation on the water status of living *Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe trees by monitoring stem shrinkage, a physiological indicator linked to tree water stress. Stem shrinkage occurs as water is withdrawn primarily from the xylem, but also from phloem tissues and internal storage pools to sustain canopy transpiration, while night-time swelling reflects water uptake and stem refilling (Zweifel et al., 2016). Among stem shrinkage metrics, tree water deficit (TWD) and maximum daily shrinkage (MDS) are continuous, high-resolution parameters used to assess tree water status and serve as indicators of water stress (Peters et al., 2025). In this study, these complementary parameters were applied to capture long-term cumulative changes (TWD) and short-term diurnal fluctuations (MDS), enabling the evaluation of mistletoe-induced water stress in Anatolian black pine under natural field conditions.

We hypothesized that: (i) mistletoe-infested trees would exhibit higher TWD and MDS values than non-infected trees, and (ii) water status in infected trees would be more strongly influenced by meteorological fluctuations than in non-infected trees.

2. Materials and methods

2.1. Location of the study area and stand characteristics

The research site is located within the Aziziye/Burdur forest district in the southwestern Mediterranean region of Türkiye (37°25'05"N, 30°17'03"E; 1480 m above sea level; Figure 1). The study area has an average slope of 15° and is predominantly north-facing. The climate of the region is characterized as typically Mediterranean, featuring dry summers and mild, rainy winters, with semi-arid traits (C1) as indicated by the Thornthwaite climate classification index. A total of five mistletoe-infected and four non-infected Anatolian black pine [*Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe] trees were selected as representative individuals of the forest stand to monitor stem radius change (SRC). Non-infected trees were chosen based on smooth trunks, intact canopies, and the absence of mistletoe infection or other diseases. To minimize variability, infected and non-infected trees were selected within 800 m² of the study area, ensuring similar height and diameter at breast height (DBH, 1.3 m). Tree age was determined at DBH using an increment borer (Haglöf, Sweden). The infected trees had an average DBH of 28.65 ± 2.28 cm, average height of 10.9 ± 0.66 m, and mean age of 83.2 ± 7.9 years. In comparison, non-infected trees had an average DBH of 28.56 ± 8.0 cm, average height of 11.3 ± 0.51 m, and mean age of 80.5 ± 13.6 years.

2.2. Meteorological observations

Data on air temperature (Tair, °C), relative humidity (RH, %), precipitation (Pp, mm), and global radiation (GR, W m^{-2}) were collected using Minikin RTHi and Minikin ERi automated meteorological instruments (EMS Brno, Czechia). These instruments, installed 2 meters high in an open area 3.7 km from the study area (Figure 1), recorded data every 5 minutes, which were later averaged into hourly and daily values. Air vapor pressure deficit (VPD, kPa) was derived from air temperature and relative humidity measurements. Soil water potential (SWP, Ψ, bar) was monitored at three depths (10, 25 and 50 cm) in two locations under the forest canopy. Hourly readings were taken using three calibrated gypsum blocks per location (Delmhorst Inc., USA) and recorded with a Microlog SP3 data logger (EMS Brno, Czechia). Daily SWP values were calculated as the mean of all six gypsum block readings.

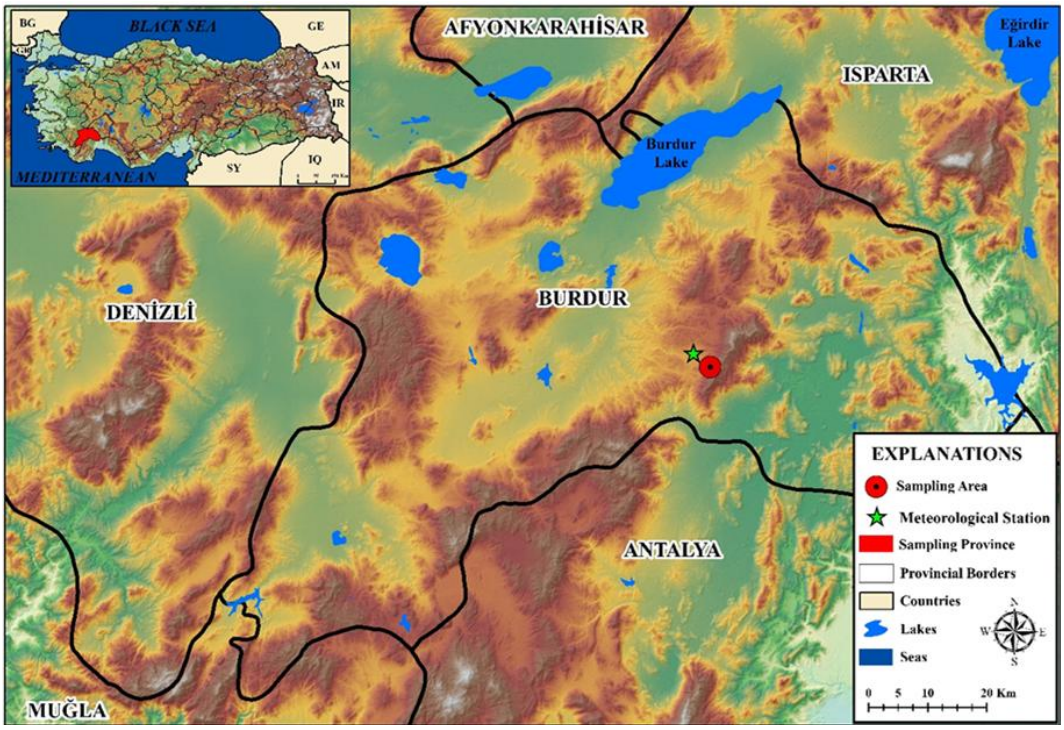

Figure 1. The location of the study area and the meteorological station in the Aziziye, Burdur/ Türkiye

2.3. Determining mistletoe density on trees

A six-level dwarf mistletoe infection classification system was used to determine mistletoe density on infected trees (Hawksworth, 1977). In this system, the live crown is divided into three equal sections, and mistletoe density is calculated for each section by assigning the following values: 0 for no infection, 1 for an infection rate below 50%, and 2 for an infection rate above 50%. The values for the three sections are then summed to determine the overall infection level for each tree. The classification is as follows: 0 indicates no infection, 1–2 indicates low infection, 3–4 indicates moderate infection, and 5–6 indicates high infection. According to the infection classification system described above, all of the infected sample trees in this study were moderately infected (3).

2.4. Extraction of tree water deficit and maximum daily shrinkage from dendrometer data

Stem radius changes (SRC) were monitored every 10 minutes during the 2024 growing period (April 1st – November 15th) using automatic band dendrometers (DR 26C, EMS Brno, Czechia; accuracy: <1 µm). Instruments were installed 3 meters high, parallel to the slope, with data recorded in the internal data loggers of each dendrometer. To ensure accuracy, the bark was carefully thinned without damaging the living tissue, thereby minimizing the influence of bark swelling and shrinking on the measurements.The 10-minute interval data were averaged into 1-hour measurements for further analysis. To improve replication in the analysis, data from sample trees were averaged according to tree infestation status: VA+ (mistletoe-infected trees) and VA- (non-infected trees) (Figure 2).

The stem radius changes (SRC) recorded by dendrometers include both permanent stem growth (driven by cambial activity and cell expansion) and short-term fluctuations due to water deficit, causing shrinkage and swelling (Zweifel et al., 2005; Deslauriers et al., 2007). To isolate tree water deficit (TWD, µm h^{-1}), raw dendrometer data were de-trended using the "zero growth concept" (Zweifel et al., 2016), which defines TWD as the difference between the most recent maximum stem radius and the current stem radius. TWD was calculated using raw dendrometer data via the “dendRoAnalyst” package (Aryal et al., 2020) in R version 4.2.2. Maximum daily shrinkage (MDS, µm d^{-1}), defined as the difference between the daily maximum and minimum stem radius, was extracted using Microsoft Excel. TWD reflects the long-term imbalance between water uptake and water usage, indicating cumulative water stress. In contrast, MDS quantifies diurnal stem contraction, representing daily water usage due to transpiration and water withdrawal from stem tissues (Güney et al. 2020a).

2.5. Statistical analyses

The statistical analyses that followed were executed in R version 4.2.2. Initially, the data were subjected to a normality test using the Kolmogorov-Smirnov method (p<0.05). To evaluate the correlation between environmental factors and TWD/MDS over the study period, Spearman non-parametric correlation coefficients were calculated for climate variables, i.e. Pp, Tair, RH, VPD, GR, SWP, GRO, and TWD/MDS using the R package "correlation" (Makowski et al., 2020).

The comparison of TWD values and MDS values between infected and non-infected trees was conducted using a Mann-Whitney U test, facilitated by the “dplyr” package in R (Wickham et al., 2022), with a significance level set at p<0.05.

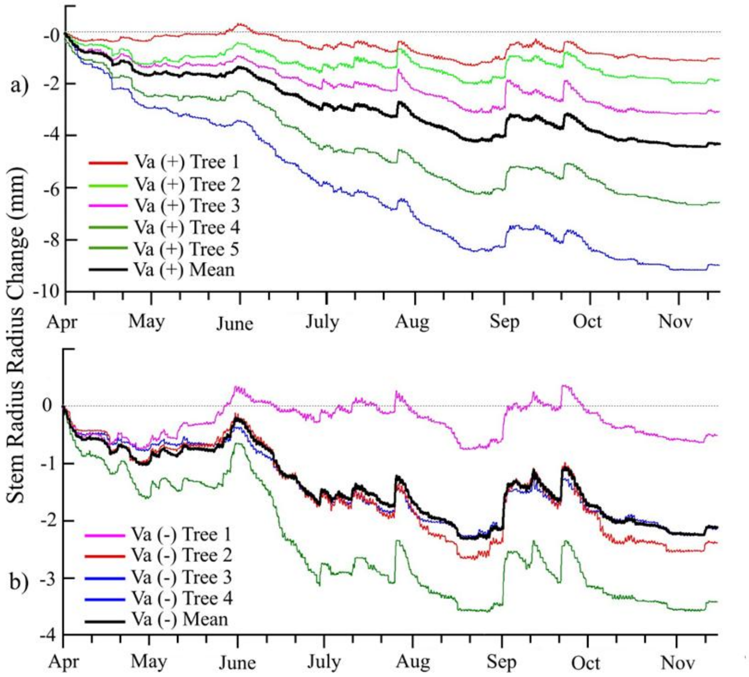

Figure 2. Hourly stem radius change (SRC) of the sample trees in April 1st to November 15th in 2024. a: Mistletoe (*Viscum album* subsp. *austriacum*) infected trees (VA+) and b: non-infected trees (VA-). The black lines in graphs represent the calculated mean SRC for both infected and healthy trees.

3. Results

3.1. Environmental conditions during the study period

During the vegetation period from April 1st to November 15th, 2024, meteorological measurements conducted in the study area indicated that air temperature ranged from a maximum of 35.5°C in June and a minimum of -3.7°C in November to (Figure 3c, Table 1). The mean air temperature over this period was recorded as 16.6°C. Total precipitation during the study period amounted to 196.4 mm (Figure 3d). The average relative humidity (RH) and vapor pressure deficit (VPD) were 57% and 1.12 kPa, respectively (Figure 3b, Figure 3f). Comparisons with long-term climatic data revealed that total precipitation was approximately 23% (46.2 mm) lower than the 31-year (1993-2023) mean for the same period of the year (Table 1).

The soil water potential remained between 0 and -2 bar until May 10, indicating a high level of soil moisture during this period. Following this date, a rapid decline in soil water potential was observed. Although intermittent rainfall events led to temporary increases in soil water potential, it ultimately reached the wilting point (-15 bar) during June, July, and August. From the first week of October until the end of the study on November 15th, the soil water potential remained consistently at the wilting point (Figure 3e).

3.2. Tree Water Deficit and Maximum Daily Shrinkage Dynamics

The analysis of TWD values revealed that although mistletoe-infected and non-infected trees exhibited similar temporal patterns throughout the study period, their absolute values differed significantly (Figure 4a). This discrepancy was most pronounced during the summer months, when drought stress peaked, and in October-November, when soil water potential consistently remained at the wilting point (Figure 3e). Results showed that neither infected nor non-infected trees fully rehydrated during the study period. As a result, no stem increment was observed in either group (Figure 2). The average TWD value for infected trees was 4455.8 ± 1853.1 µm, compared to 2314.8 ± 991.9 µm for non-infected trees (Figure 5), indicating a 92% higher TWD in infected trees. The Mann-Whitney U test confirmed that this difference was statistically significant ($p<0.05$), emphasizing the strong impact of *Viscum album* infection on tree water deficit.

Similarly, MDS values consistently showed greater stem shrinkage in mistletoe-infected trees (VA+) than in non-infected trees (VA-) throughout the measurement period (Figure 4b). The average MDS value for infected trees was 134.9 ± 85.4 µm, while for non-infected trees, it was 119.5 ± 80.9 µm, reflecting a 12% higher MDS for infected trees. This difference was also statistically significant ($p < 0.05$), emphasizing the pronounced effect of *Viscum album*.

Table 1. Mean values (± Standard deviation) and long-term averages of climate variables measured at the study area. SD: Standard deviation.

Variable	2024 (April 1st to November 15th)		Long Term Average (April 1st to November 15th)
	Mean±SD	Range	
Air temperature (°C)	16.6.±8.2	-3.7-35.5	18.1
Global radiation (W m^{-2})*	360±220	3.0-1062.9	Not available
Relative humidity (%)	57 ± 25.3	6.9-100	51.9
Total Precipitation (mm)	196.4	0-12.8	242.6
Soil water potential (bar)	-7.8±5.8	-0.2 – (-15)	Not available

* Global radiation in the column represents the mean daytime radiation.

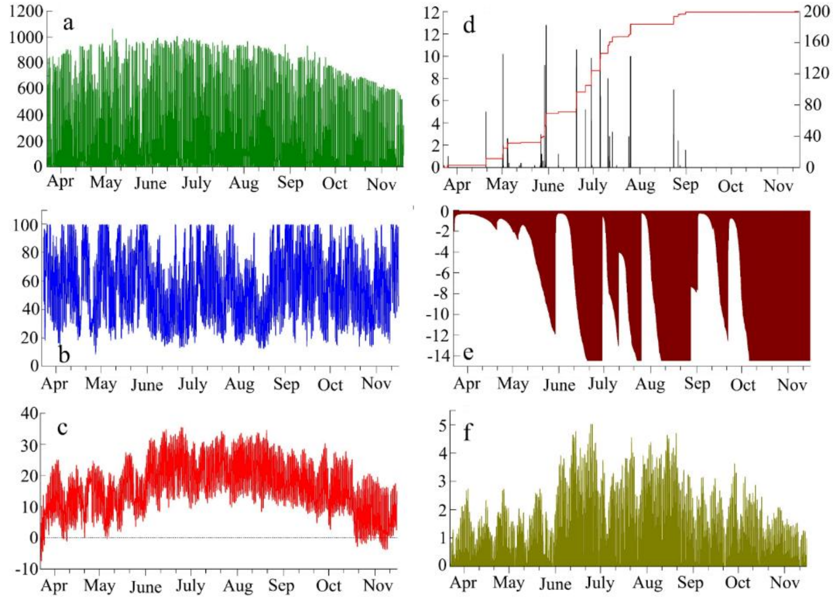

Figure 3. Climate conditions between April-November 2024 in the study area. a) Global radiation (GR, W m^{-2}), b) relative humidity (RH, %), c) air temperature (Tair, °C), d) precipitation events (Pp, mm, black bars) and cumulative precipitation (mm, red line), e) soil water potential (Ψ, bar), f) vapor pressure deficit (VPD, kPa).

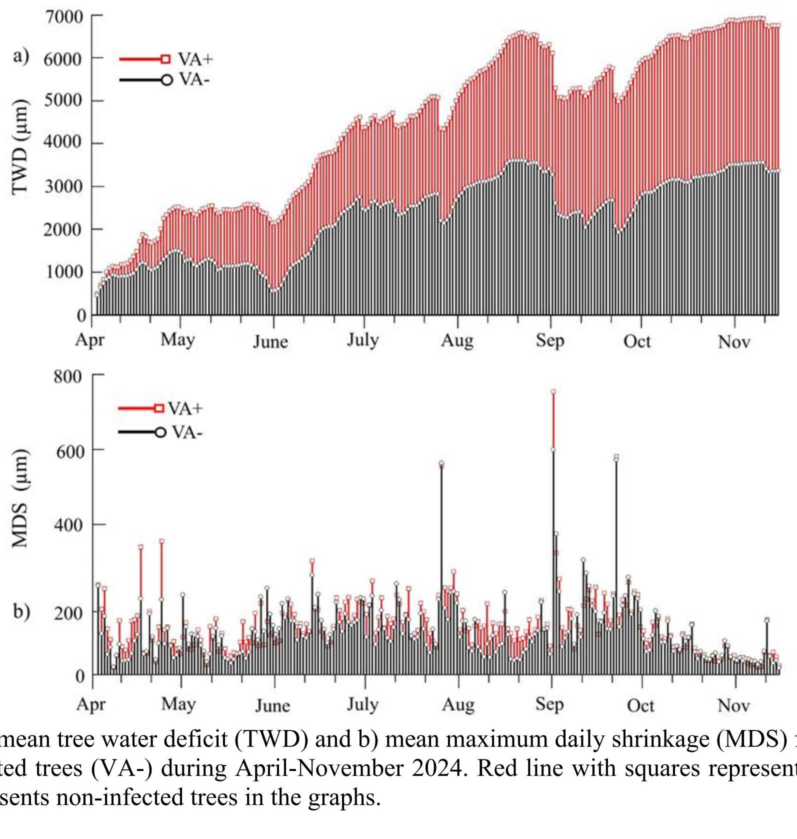

Figure 4. a) trends in mean tree water deficit (TWD) and b) mean maximum daily shrinkage (MDS) for mistletoe infected trees (VA+) and non-infected trees (VA-) during April-November 2024. Red line with squares represents infected trees and black line with circles represents non-infected trees in the graphs.

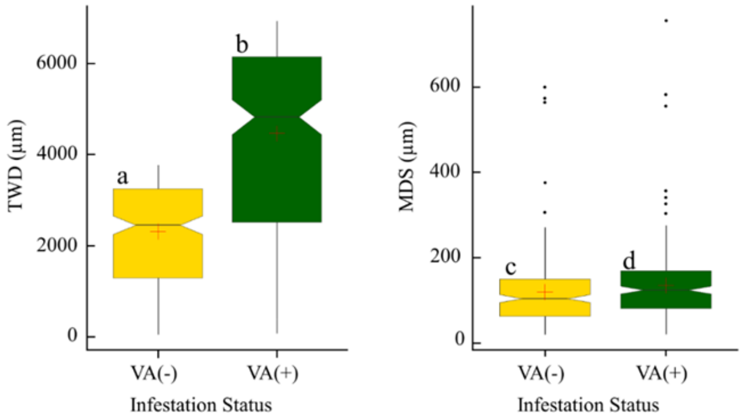

Figure 5. Box plots for average TWD (tree water deficit) and average MDS (maximum daily shrinkage) of *Viscum album* infected (green, VA+) and non-infected (gold, VA-) trees during April-November 2024. The values represented by distinct letters show significant differences according to the Mann-Whitney U test ($p < 0.05$). In each box plot, data are plotted as median and percentiles (box, 25th and 75th percentiles). Additionally, means were plotted as red crosses.

3.3 Impact of Environmental Factors on Tree Water Deficit and Maximum Daily Shrinkage

The analysis of the relationship between average TWD in infected and non-infected trees and climatic variables indicated that SWP exhibited the strongest negative correlation with both infestation categories. Additionally, the correlation coefficients for the two tree infestation categories were comparable (Table 2).

Among all examined factors, air temperature showed the strongest association with average MDS values in both infected and non-infected trees (Table 2). However, an inconsistency was observed in the effect of precipitation on MDS values, as it significantly influenced the MDS of non-infected trees (p<0.01) but did not have a substantial impact on infected trees. Overall, meteorological variables had a stronger impact on the MDS values of infected trees compared to non-infected trees.

4. Discussion

4.1. Impact of Mistletoe Infestation on Tree Water Status Dynamics

Mistletoes are semi-parasitic plants that extract water and mineral nutrients from their host due to the absence of a root system (Muche et al., 2022), significantly impacting host tree water balance (Yang et al., 2017). Our results showed that mistletoe-infected trees had significantly higher TWD and MDS values, suggesting greater water stress (Figures 4 and 5).

These findings align with previous research demonstrating mistletoe-induced water stress across various tree species. For instance, Kubov et al. (2020) reported a 1 MPa decrease in leaf water potential and a 14% decline in water use efficiency in sessile oak (*Quercus petraea*) due to mistletoe infestation. Similarly, Raftoyannis et al. (2015) found that mistletoe in Grecian fir (*Abies cephalonica)* lowered midday leaf water potential by 0.10 to 0.40 MPa, and Mutlu et al. (2016) observed that mistletoe-infected Scots pine (*Pinus sylvestris)* needles contained 15% less water in summer than those of non-infected trees.

The elevated TWD and MDS values in infected trees can be attributed to mistletoe ecophysiology and its disruptive effects on host water regulation. Mistletoes maintain a lower leaf water potential than their hosts (Garkoti et al., 2002; Cocoletzi et al., 2020; Amutenya et al., 2023), enabling them to continue transpiration even when the host closes its stomata to prevent cavitation during droughts. This conflicting water regulation system (Glatzel and Geils, 2009) results in excessive water loss from the host tree. By maintaining a lower leaf potential, mistletoes can alter the transpiration pathway (Griebel et al., 2022).

Table 2. Spearman's correlation coefficients between daily means of TWD, MDS, and daily means of climate variables in 1st April-15th November 2024.

Variable	GR	Tair	RH	SWP	P_p	VPD	Soil Temp
MDS VA+	0.4***	**0.50*****	-0.08	-0.32***	0.05	0.4***	0.45***
MDS VA-	0.28***	**0.36*****	0,06	-0.34***	0.18**	0.22***	0.35***
TWD VA+	-0.40***	-0.05	-0.07	**-0.78*****	-0.15**	0.30***	-0.03
TWD VA-	-0.28***	0.06	-0.15*	**-0.73*****	-0.08	0.19***	-0.07

GR: global radiation, W m^{-2}. Tair: daily mean air temperature, °C. RH: air relative humidity, %. SWP: soil water potential, bar. P_p: precipitation, mm. VPD: vapour pressure deficit, kPa. VA+ *Viscum album* infected trees, VA-: non-infected trees. The significance levels were established as *p< 0.05, **p < 0.01, and ***p< 0.001. The highest correlation for each dependent variable is emphasized in bold. n= 229 days.

Recent evidence indicates that mistletoe infection disrupts host tree hydraulics across multiple organizational scales. Gas-exchange measurements have shown that mistletoes maintain persistently higher stomatal conductance and transpiration rates than their hosts, diverting a disproportionate share of xylem water to the parasite (Mathiasen et al., 2008; Ullmann et al., 1985). At the needle level, increasing infection severity progressively reduces needle cross-sectional and mesophyll areas while increasing stomatal density and decreasing stomatal size-traits indicative of acclimation to chronic water limitation (Öztürk et al., 2022). In Scots pine, *Viscum album* infestation significantly decreases tracheid wall thickness and lumen diameter, and reduces vascular tissue area in needles, directly impairing both hydraulic conductivity and photosynthetic capacity (Öztürk et al., 2019). Whole-tree assessments further show that mistletoe infestation flattens the leaf-area-to-sapwood-area relationship (Bilgili et al., 2020), suggesting that the conductive sapwood becomes increasingly inadequate to support the foliage. Moreover, because mistletoe leaves tend to close their stomata slower than those of their hosts (Sala et al., 2001; Zweifel et al., 2012; Urban et al., 2012; Griebel et al., 2022), even moderate levels of infection can predispose branches and entire crowns to hydraulic failure during drought conditions.

Moreover, mistletoe foliage is succulent, providing substantial water-storage capacity and allowing faster rehydration than host leaves. This sustained water withdrawal delays host recovery following dry periods (Glatzel and Geils, 2009). During the exceptionally dry 2024 season (23 % below the long-term mean precipitation), both infected and non-infected Anatolian black pine trees ceased radial growth (Figure 2). However, stem-dendrometer records showed greater maximum daily shrinkage and more persistent tree-water deficit in infected individuals, indicating that the host's isohydric stomatal closure was undermined by the parasite's unregulated water loss and thereby heightened the risk of xylem cavitation.

4.2. Environmental control on water status dynamics in infected and non-infected trees

Projected climate change in the Mediterranean Basin underscores the importance of understanding tree water stress drivers, particularly in mistletoe-infected trees, which may respond differently to environmental variables. This knowledge is crucial for predicting the impact of a drier climate on forest ecosystems.

Our study found that soil water potential (negatively), global radiation, and vapor pressure deficit (VPD) were the most influential factors affecting tree water deficit (TWD) in both non-infected and infected trees (Table 2). Maximum daily shrinkage (MDS) in non-infected trees correlated most with air temperature, soil temperature, and soil water potential, whereas in infected trees, air temperature, VPD, global radiation, and soil water potential were the dominant factors.

Previous studies have extensively explored the relationship between TWD and meteorological factors. Drew et al. (2011) found that in Cypress pine (*Callitris intratropica)*, TWD is mainly driven by soil water availability, along with air temperature and relative humidity. Similarly, Zweifel et al. (2016) reported that TWD in *Pinus, Picea, Fraxinus,* and *Fagus* species is strongly influenced by VPD and soil water potential. Perron et al. (2024) highlighted the role of radiation, air temperature, and soil water availability in long-term TWD trends in Canada's boreal forests, while Dunkleberger et al. (2023) identified soil water availability, VPD, and wind speed as key drivers of TWD in Contorta pine (*Pinus contorta)* and white spruce (*Picea glauca)*.

These studies collectively indicate that any factor increasing evapotranspiration also increases TWD, though its relative importance varies by species and location. Higher VPD enhances transpiration when stomata are open, leading to greater TWD (Grossiord et al., 2020). Solar radiation raises air and leaf temperatures, increasing the need for cooling via transpiration (Pieruschka et al., 2010). Wind disrupts the leaf boundary layer, accelerating water loss (Shapira et al., 2024). Additionally, when soil water availability is insufficient to meet increasing evaporative demand, TWD intensifies (Bachofen et al., 2024).

Our findings confirm that TWD is primarily influenced by soil water availability, radiation and VPD. In our study area, strong evaporative demand resulted from high air temperatures and solar radiation, yet soil water availability was the most limiting factor, exhibiting the strongest negative correlation with TWD. Notably, TWD in infected trees was more strongly influenced by changes in VPD and radiation than in non-infected trees (Table 2). This may be because non-infected trees reduce water transport by closing their stomata in response to high evapotranspiration demand, whereas mistletoe-infected trees, despite stomatal closure, continue to experience water loss due to the transpiration of mistletoe itself. This results in a stronger correlation between TWD and these environmental factors.

Similarly, our study found that MDS in both non-infected and infected trees was most correlated with air and soil temperature, VPD, and global radiation. Our results align with previous research indicating that temperature is the most influential climatic variable on MDS, with GR and VPD also playing significant roles (Ortuño et al., 2006; Conejero et al., 2007; Güney et al., 2020b). Like TWD, MDS is largely driven by factors that increase evaporative demand, particularly air temperature.

Mistletoe-infected trees appeared more sensitive to environmental changes and exhibited higher MDS values than non-infected trees (Table 2). This may be due to the greater water storage and higher hydraulic capacity in the tissues of non-infected trees, which could reduce the correlation between tree water status parameters and environmental changes. In 2024, precipitation in the study area was sparse, with most events remaining below 10 mm (Figure 3d). While rainfall events would normally be expected to help rehydrate trees and reduce MDS (Glatzel and Geils, 2009), such a response was evident only in non-infected trees, whereas mistletoe-infected trees showed no significant reaction. Another potential factor is that infected trees may have closed their stomata in response to high evaporative demand; however, they might have continued to experience greater water loss due to the ongoing transpiration of the mistletoe itself. This suggests that mistletoe-infected trees are less capable of utilizing limited water inputs for recovery, potentially exacerbating their water stress under prolonged dry conditions. One possible explanation is that, unlike non-infected trees, infected trees struggle to utilize small rainfall events due to the mistletoe's ability to rehydrate rapidly.

5. Conclusion

Our study indicates that mistletoe infection significantly alters the water status of host Anatolian black pine trees, making them more sensitive to environmental variations and leading to higher tree water deficit (TWD) and maximum daily shrinkage (MDS) compared to non-infected trees. While rainfall events were expected to aid tree rehydration and influence MDS, infected trees showed no significant response, suggesting a reduced ability to utilize limited precipitation. Stronger correlations between climatic factors and TWD/MDS further emphasize the disruption of water regulation in infected trees. These findings underscore the potential role of mistletoe contributing to drought-related stress in Anatolian black pine.

Since the Mediterranean region is projected to become hotter and drier, and even non-infected trees are already experiencing drought stress severe enough to limit their growth, mistletoe-infected trees are likely at an even greater risk of mortality. Considering that healthy forest stands play a crucial role in regulating hydrological processes within watersheds, the deterioration of water status and vitality in mistletoe-infected trees may impair canopy transpiration, soil moisture regulation, and overall watershed water balance. As a result, mistletoe control measures are recommended in the study area and other regions with similar environmental conditions to mitigate its impact on tree health and survival.

To further investigate the effects of mistletoe on the physiological mechanisms of its host and its long-term ecological impacts on forest resilience, future research should incorporate measurements of sap flow, leaf and stem water potential changes, and stomatal conductance to provide deeper insights into the ecohydrological consequences of mistletoe infestation.

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