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The effects of mistletoe (*Viscum album* L.) on the physiological properties of some drupe trees in Turkey

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Abstract: In the present study, the effects of mistletoe (*Viscum album* L.) on the physiological properties of drupe trees were investigated. In order to achieve this goal, sulfhydryl, chlorophyll a and b, total chlorophyll, ascorbic acid, total amino acid, proline, carotenoid, glucose, and fructose levels, as well as leaf proportional water contents were determined in both mistletoe and its hosts under laboratory conditions. According to the results, mistletoe caused a significant decrease in water content and reduced total ascorbic acid content in apricot, almond, and plum. Additionally, mistletoe did not affect the total amino acid, glucose, and fructose concentrations of the hosts. Carotenoid and total chlorophyll contents, on the other hand, were statistically similar in the control and hosts in almond and apricot. It was also observed that mistletoe had virtually no effect on the amount of chlorophyll a in all hosts; however, it affected chlorophyll b content. As a semiparasite, mistletoe caused branches to drift backwards from the tip of their shoots by using the water and other nutritional elements of the host tree, as well as clogging their xylems with its haustorium. In addition, mistletoe acts as a stress factor, increasing host proline and sulfhydryl levels and, thereby, causing the branches to drift backwards.

Key words: Mistletoe (*Viscum album* L.), drupe, proportional water, chlorophyll, carotenoid, proline

1. Introduction

Fruit production is an important branch of horticulture, as fruits have been used not only for nutrition but also to meet personal and social needs (Ercisli et al., 2008). Drupe production in countries around the world, including Turkey, has important potential. Turkey ranks 1st in the world in apricot production with annual production at 985,000 t, Italy ranks 2nd with 266,372 t, and Algeria ranks 3rd with 256,890 t. In terms of almond production, the USA ranks 1st with 1,029,655 t, and Turkey ranks 5th with 90,000 t. China ranks 1st in plum production with 6,804,399 t, and Turkey ranks 5th with 291,934 t (Faostat, 2017).

Mistletoe (*Viscum album* L.) is a semiparasitic plant, and it has been known to reduce the quality and yield of fruits in various parts of the world, including Turkey. Three subspecies of Viscum genus, which is categorized under the family Santalaceae of Santalales, are: *V. album* L., *V. album* L. sp. *abietis* (Wiesb.) Abrom., and *V. album* L. sp. *austriacum* (Wiesb.) Vollm. This semiparasite is a subtype containing chlorophyll a and b in its leaves and is (Kew, 2019) able to perform photosynthesis, as first reported by Miller (1982). Although it is capable of synthesizing its own glucose, it still requires water and nutrients from the host and generates its own metabolites during photosynthesis. The semiparasite uses the water and organic substances of the host, taken in through its haustorium from the xylem, initially weakening and eventually killing the host. Of all biological stress sources, mistletoe is considered one of the most destructive for plants (Fischer, 1983; Ehleringer et al., 1985; Hawksworth and Scharpf, 1986; Hawksworth and Wiens, 1996; Watson, 2001; Zuber, 2004; Zuber and Widmer, 2009; Türe et al., 2010). In Turkey, the most subspecies of mistletoe display semiparasitic features, and they can live on a variety of fruit trees, including, but not limited to; apricot, apple, pear, wild pear, and hawthorn. In Turkey, the most common fruit tree hosts on which mistletoe was observed were Ankara pear, Braeburn apple, almond, apricot, and plum. The disease caused by mistletoe was most severe in *Amygdalus* spp. (48.54%), followed by *Prunus armeniaca* L. (34.98%), and *Pyrus communis* L. (28.64%) (Üstüner, 2003; Üstüner et al., 2015). However, when the sugar content of mistletoe (glucose, fructose, and sucrose) on populus was evaluated, it was higher in spring than in autumn. While the xylem of populus contained the highest amount of carbohydrate in spring, concentrations of the same molecules were significantly lower in autumn and summer (Escher et al., 2004). Additionally, when

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Ziegler et al. (2009) measured the rates of transpiration and stem flow, they found that the values were higher in mistletoe than in their woody hosts.

The present study aims to investigate the effects of mistletoe (*V. album* L.) on chlorophyll a and b, proline, carotenoid, total chlorophyll, ascorbic acid, total amino acid, sulfhydryl, fructose, and glucose levels and proportional water contents of drupe (almond, apricot, and plum) grown in the Central Anatolian region of Turkey.

2. Materials and methods

2.1. Materials

The biological materials used in the study [apricot, almond, plum, and mistletoe (*V. album* L.) leaves] were collected from the Central Anatolian region of Turkey (Niğde, Nevşehir, and Kayseri Provinces) between 2014 and 2017.

2.2. Climatic characteristics of research area

According to meteorological data from the region, the annual mean temperature was 19.20 °C, relative humidity 46.39 g/m^3, sun duration 8.14 h/day, mean wind speed 2.941 m/s, mean precipitation 0.966 mm, and surface soil temperature 4.235 °C throughout the study.

2.3. Soil properties of the research area

The soil of the study area was analyzed, and it is arid, sandy, and slightly alkaline. Total salinity levels were relatively low. The phosphorous, organic matter, and potassium contents of the soil were 3.2 mg/kg, 0.41%, and 43 mg/kg, respectively. The pH and lime ratio were 7.5 and 7.3, respectively.

2.4. Methods

Analyses were made in the laboratory of the Department of Biology, Faculty of Science, at Çukurova University and in the USKIM laboratory of Kahramanmaraş Sütçü İmam University. The methods described by Sairam et al. (2002) were used to calculate proportional water content and chlorophyll a and b and carotenoid levels. For the estimation of other parameters, the protocol defined by Lichtenthaler and Wellburn (1983) was used. Additionally, the SH groups were analyzed using 5-5 dithiobis (2-nitrobenzoik acid; DTNB) solution prepared in a 5% metaphosphoric acid extraction. Finally, the reduced and total ascorbic acid amounts were measured along with proline and other amino acid levels, as previously reported (Spies, 1957; Bates et al., 1973; Cakmak and Marschner, 1992).

2.5. Statistical analysis

For the purpose of revealing the statistical significance of differences among the results for each parameter, in both mistletoe and its hosts, the SPSS 20 software package was used for variance analyses. Duncan and ANOVA tests were performed to examine the dissimilarities between means. For values where $P < 0.05$, the difference was considered statistically significant.

3. Results

3.1. Leaf proportional water content

Among the studied drupe trees, almond, apricot, and plum showed statistically higher levels of leaf proportional water content in the mistletoe, compared to control and host plants (Table 1), while the mistletoe had reduced 7.5%–12.2% of leaf proportional water content of the host, compared to the control.

3.2. Chlorophyll a and b

Statistically, chlorophyll a was not significantly affected, while chlorophyll b was affected by mistletoe in all hosts. In the infected samples, however, mistletoe only influenced chlorophyll b content of the host and did not seem to influence chlorophyll a (Table 2). Mistletoe reduced about 2.19–16.1 (mg/g) of chlorophyll b content in almond, apricot, and plum.

3.3. The carotenoid and total chlorophyll content

In all hosts, the carotenoid and total chlorophyll contents of mistletoe were lower than those in control and hosts. The

Table 1. Proportional water content (%) of control, hosts, and mistletoe.

	Proportional water content (%)			
Fruit trees	Control	Host	Mistletoe	F and P
Almond	45.5 ± 0.27 B	38.02 ± 6.0 C	92.4 ± 3.2 A	$F_{2,12} = 10.920$ $P < 0.0001$
Apricot	52.08 ± 0.68 B	40.0 ± 2.6 C	96.8 ± 5.0 A	$F_{2,12} = 2796.8$ $P < 0.0001$
Plum	37.7 ± 0.3 B	25.5 ± 2.4 C	62.5 ± 6.0 A	$F_{2,12} = 7242.6$ $P < 0.0001$

One-way analysis of variance (ANOVA) and DUNCAN tests were applied to the data, and the differences between the means were calculated at the significance level of $P < 0.05$.

Table 2. Chlorophyll a and b contents of control, hosts, and mistletoe.

	Chlorophyll a content (mg g^{-1})				Chlorophyll b content (mg g^{-1})			
Fruit trees	Control	Host	Mistletoe	F and P	Control	Host	Mistletoe	F and P
Almond	8.84 ± 0.61 A	9.91 ± 1.34 A	3.03 ± 0.29 B	$F_{2,6}$ = 18.26 P < 0.01	4.79 ± 0.4 A	2.6 ± 0.47 B	0.67 ± 0.17 C	$F_{2,6}$ = 30.92 P < 0.01
Apricot	10.31 ± 0.38 A	9.35 ± 1.61 A	4.71 ± 0.86 B	$F_{2,6}$ = 183.2 P < 0.0001	3.35 ± 0.2 A	2.34 ± 0.97 B	1.46 ± 0.28 C	$F_{2,6}$ = 2.82 P = 0.1368
Plum	20.65 ± 1.03 A	22.9 ± 0.48 A	5.81 ± 0.35 B	$F_{2,6}$ = 7.74 P < 0.05	26.12 ± 3.5 A	10.02 ± 0.54 B	1.33 ± 0.13 C	$F_{2,6}$ = 36.10 P < 0.001

carotenoid and total chlorophyll contents were statistically similar in control and hosts for almond and apricot (Table 3). Mistletoe reduced the carotenoid and total chlorophyll contents of the host at a statistically significant level in plum.

3.4. Proline and total amino acid levels

Proline concentration in mistletoe and hosts were higher than in control for all fruits (Table 4). Mistletoe increased the amount of proline in all hosts 0.5%–0.6%. Mistletoe, a biologic stress factor, caused proline content of hosts to increase.

Mistletoe decreased total amino acid levels in three drupe. However, while mistletoe had a lower amount of total amino acids by number, statistically, it was in the same group as the control and hosts (Table 5). In all cases, however, mistletoe had no influence on total amino acid content of the hosts.

3.5. Reduced and total ascorbic acid concentrations

Reduced and total ascorbic acid concentrations of hosts were lower than in control (Table 6). Mistletoe had a negative effect on reduced and total ascorbic acid in all hosts. This effect on hosts was 0.04%–0.24% and 0.04%–0.09%, respectively.

3.6. Glucose and fructose contents

Mistletoe displayed lower concentrations of glucose and fructose than the control and host in three fruit species (Table 7). Mistletoe reduced the amount of glucose and fructose in all hosts as a numerical value; however, it was statistically significant in the same group.

3.7. Sulfhydryl concentrations

When sulfhydryl (SH) concentrations of the semiparasite were measured, mistletoe and the hosts had higher levels than control in the three fruits (Table 8). Mistletoe is a biologic stress factor, and the sulfhydryl concentration increased in all three hosts. This increase was 0.07%–0.09%.

4. Discussion

Through these investigations, it was determined that mistletoe had 2–3 times higher leaf proportional water content than the hosts and control. Leaf proportional water content was reduced 7.5%–12.2% in the host compared to the control. These results were in accordance with Hawksworth and Wiens (1996), who showed that mistletoe had levels up to 5 times greater compared to the host. Mistletoe most successfully competes for a share of the host's water. Previous studies have shown that that the transpiration rate of mistletoe on trees was 3-fold higher than in the host; particularly when calculated according to leaf surface. Proportional water content of mistletoe was greater than in all fruit trees regardless of their status in terms of mistletoe infection (Schulze et al., 1984; Hosseini et al., 2008; Glatzel and Geils, 2009; Oyetunji and Edagbo, 2013; Murugan et al., 2014; Üstüner and Düzenli, 2017).

According to our findings, mistletoe did not affect chlorophyll a content of the host, while it affected chlorophyll b content in all hosts. Mistletoe is capable of producing its own metabolites during the process of photosynthesis. It has been previously shown that mistletoe has significantly lower levels of chlorophyll a compared to its hosts (Zuber, 2004; Chatterjee and Ghosh, 2008). In the current study, mistletoe had higher amounts of chlorophyll a than chlorophyll b, although Oyetunji and Edagbo (2013) concluded that both the host (citrus and irvingia) and African mistletoe (*Tapinanthus bangwensis*) possessed greater chlorophyll b content. In another study, mistletoe had lower chlorophyll b content than the control, and yet its levels were similar to the host for Braeburn apple (Üstüner and Düzenli, 2017). In the same study, when the comparison was made in Ankara pear and hawthorn, the results were similar to the control and the host.

Mistletoe had a very limited effect on carotenoid and total chlorophyll contents of the host. However, mistletoe reduced the carotenoid and total chlorophyll contents of the host at a statistically significant level in plum. Mistletoe contains carotenoids and all of the pigments, including chlorophyll a and b, that are required for the synthesis of glucose using light (Becker, 2000). Oyetunji and Edagbo (2013) suggested that the total chlorophyll content of mistletoe was significantly lower than in citrus, while no

Table 3. The carotenoid and total chlorophyll contents of hosts and mistletoe.

	Carotenoid content (mg g^{-1})				Total chlorophyll content (mg g^{-1})			
Fruit trees	Control	Host	Mistletoe	F and P	Control	Host	Mistletoe	F and P
Almond	4.29 ± 0.38 A	2.45 ± 1.25 A	1.99 ± 0.11 B	$F_{2,6}$ = 19.91 P < 0.01	13.63 ± 1 A	12.5 ± 1.81 A	3.70 ± 0.45 B	$F_{2,6}$ = 2.58 P = 0.1556
Apricot	3.47 ± 0.05 A	2.56 ± 0.71 A	1.93 ± 0.36 B	$F_{2,6}$ = 5.74 P < 0.05	13.36 ± 0.6 A	13.6 ± 2.57 A	6.17 ± 1.14 B	$F_{2,6}$ = 2.84 P = 0.1355
Plum	10.76 ± 0.14 A	7.48 ± 1.87 B	2.41 ± 0.20 C	$F_{2,6}$ = 151.7 P < 0.0001	46.77 ± 2.6 A	32.9 ± 1.01 B	7.14 ± 0.48 C	$F_{2,6}$ = 17.44 P < 0.01

Table 4. Proline concentrations of control, hosts, and mistletoe.

	Proline concentration (mg g^{-1})			
Fruit trees	Control	Host	Mistletoe	F and P
Almond	0.07 ± 0.04 B	0.12 ± 0.003 A	0.13 ± 0.03 A	$F_{2,6}$ = 1.55 P = 0.2871
Apricot	0.02 ± 0 B	0.11 ± 0.01 A	0.17 ± 0.02 A	$F_{2,6}$ = 8.84 P < 0.05
Plum	0.09 ± 0.01 B	0.15 ± 0.01 A	0.22 ± 0.03 A	$F_{2,6}$ = 43.15 P < 0.001

Table 5. Total amino acid concentrations of control, hosts, and mistletoe.

	Total amino acid content (mg g^{-1})			
Fruit trees	Control	Host	Mistletoe	F and P
Almond	4.27 ± 0.3 A	3.44 ± 0.2 AB	2.81 ± 0.5 B	$F_{2,6}$ = 3.72 P = 0.0888
Apricot	4.07 ± 0.7 A	3.55 ± 0.4 A	3.40 ± 0.25 A	$F_{2,6}$ = 0.33 P = 0.7327
Plum	1.87 ± 0.88 A	1.50 ± 0.4 A	1.30 ± 0.21 A	$F_{2,6}$ = 0.33 P = 0.732

difference was observed when comparing mistletoe and irvingia. On the other hand, when the host was compared with the control plant, carotenoids increased, while total chlorophyll decreased numerically (Murugan et al., 2014). Total chlorophyll and carotenoid contents may vary according to the physiology and species of tree. The results obtained here are similar to those of the aforementioned researchers.

In the current study, proline in hosts was higher than in the control for three drupe. Mistletoe increased the proline in all three hosts. In other studies, the increase in proline concentration promoted proline oxidation, while it hindered protein synthesis (Smirnoff and Colombe, 1988). Proline is a nitrogen compound that increases under stress conditions, participates in detoxification of free O_2, and includes stress-protective properties (Bohnert and Sheveleva, 1998). Many researchers have shown that proline content increases under the effects of oxidative stress, and biological stress factors give rise to increases in proline and ascorbic peroxidase contents in apple and pear (Sairam et al., 2002; Karacif and Boyraz, 2012). Murugan et al. (2014) reported that infected plants had more proline than control plants. Mistletoe increased the amount of proline in soft-core fruit trees due to stress (Üstüner and Düzenli, 2017). The results of this study are in alignment with the above-mentioned research.

Mistletoe appeared to reduce total amino acids by a numerical value; however, statistically, it was in the same group as the control and hosts. In another study, mistletoe took the required nutrients from its host, and amino acid contents decreased in fruit trees; evidently lower leaf protein content was seen in most species of infected trees, when compared to uninfected trees (Patykowski and Kolodziejek, 2013). Although the protein content in the leaves of the mistletoe plant living on Crataegus was found to be low in the autumn season, it was found to be high in spring. The seasonal variability was not found to be significant in the mistletoe living on Acer, Populus and Robina. (Patykowski and Kolodziejek, 2016).

In this research, mistletoe reduced total ascorbic acid concentrations in all hosts. This is presumably because mistletoe takes ascorbic acid from hosts. It has been reported that some mistletoe hosts, such as apple, cherry, pear, and plum, contain ascorbic acid (Evans, 1989). Ascorbic acid content is lowered under stress conditions in some plant species and increases in others as an antioxidant defense mechanism (Sairam et al., 2005; Tiryakioğlu et al., 2006). Total and reduced ascorbic acid concentrations in hosts were lower than in control plants for Braeburn apple, Ankara pear, and hawthorn (Üstüner and Düzenli, 2017).

Table 6. Reduced and total ascorbic acid concentrations of control, hosts, and mistletoe.

	Reduced ascorbic acid (mg mL^{-1})				Total ascorbic acid (mg mL^{-1})			
Fruit trees	Control	Host	Mistletoe	F and P	Control	Host	Mistletoe	F and P
Almond	0.138 ± 0.001 A	0.134 ± 0.001 B	0.135 ± 0.001 B	$F_{2,6} = 7.42$ $P < 0.05$	0.148 ± 0 A	0.144 ± 0.001 B	0.142 ± 0.004 B	$F_{2,6} = 17.62$ $P < 0.01$
Apricot	0.161 ± 0.001 A	0.137 ± 0.0003 B	0.136 ± 0.0001 B	$F_{2,6} = 1.92$ $P = 0.2271$	0.150 ± 0.003 A	0.144 ± 0.001 B	0.137 ± 0 C	$F_{2,6} = 127.0$ $P < 0.0001$
Plum	0.138 ± 0.0009 A	0.136 ± 0.005 B	0.133 ± 0.001 B	$F_{2,6} = 4.88$ $P = 0.0552$	0.145 ± 0.003 A	0.136 ± 0 B	0.134 ± 0 B	$F_{2,6} = 103.0$ $P < 0.05$

Table 7. Glucose and fructose contents of control, hosts, and mistletoe.

	Glucose content (mg g^{-1})				Fructose content (mg g^{-1})			
Fruit trees	Control	Host	Mistletoe	F and P	Control	Host	Mistletoe	F and P
Almond	0.07 ± 0.01 A	0.04 ± 0.007 A	0.002 ± 0.0004 B	$F_{2,6} = 16.07$ $P < 0.01$	0.11 ± 0.02 A	0.105 ± 0.01 A	0.04 ± 0.006 B	$F_{2,6} = 10.38$ $P < 0.05$
Apricot	0.115 ± 0.08 A	0.09 ± 0.088 A	0.02 ± 0.007 B	$F_{2,6} = 0.54$ $P = 0.61$	0.19 ± 0.09 A	0.142 ± 0.122 A	0.05 ± 0.007 B	$F_{2,6} = 0.64$ $P = 0.5607$
Plum	0.379 ± 0.09 A	0.35 ± 0.11 A	0.002 ± 0.0009 B	$F_{2,6} = 6.07$ $P < 0.05$	0.22 ± 0.12 A	0.196 ± 0.13 A	0.08 ± 0.07 B	$F_{2,6} = 3.43$ $P = 0.667$

Table 8. Sulfhydryl (SH) concentration (ppm) of control, hosts, and mistletoe.

	SH concentration (ppm)			
Fruit trees	Control	Host	Mistletoe	F and P
Almond	0.091 ± 0.0001 B	0.098 ± 0.0002 A	0.096 ± 0.0002 A	$F_{2,6} = 10.39$ $P < 0.05$
Apricot	0.094 ± 0.0001 B	0.098 ± 0.008 A	0.097 ± 0.0002 A	$F_{2,6} = 29.36$ $P < 0.001$
Plum	0.091 ± 0.0003 B	0.099 ± 0.0003 A	0.096 ± 0 A	$F_{2,6} = 211.2$ $P < 0.0001$

The findings obtained in previous research were similar to the current results.

Similarly, glucose and fructose levels decreased in hosts with mistletoe; however, the decrease was statistically insignificant. Mistletoe needs only a small amount of glucose and fructose produced by the host plant since the parasite is able to produce these on its own, which may explain these results. One study showed that the semiparasite depends on the host for a little sugar supply (Oyetunji and Edagbo, 2013). In a study by Murugan et al. (2014), a mango plant infected by the parasite *Dendrophthoe falcate* had a reduction in soluble sugar content. Under parasitic stress conditions, total soluble sugar content decreased considerably. Glucose and fructose concentrations in hosts were lower than in control for Braeburn apple, Ankara pear, and hawthorn (Üstüner and Düzenli, 2017). A significant decrease in total sugar content under biotic stress was detected in the infected host.

Mistletoe was discovered to cause a rise in sulfhydryl concentrations in infected hosts. Sulfhydryl content in hosts was higher than in control probably because mistletoe acts as a biological stress factor on its host. In previous studies, it was reported that the SH content of plants can be increased by low temperatures and water stress during photosynthesis. It was also determined that antioxidants may be reduced under stress conditions in various environments (Smith et al., 1985; Hodges and Forney, 2000). Mistletoe increases the amount of SH in some soft-core fruit trees (Üstüner and Düzenli, 2017).

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