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# Water relations in olive trees under cold conditions

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## ABSTRACT

A common effect of cold temperatures on plants is dehydration. When the temperature decreases below some specific thresholds, olive trees, as other sensitive crops, reduce their leaf water potential and transpiration rates even when soil water content is not limiting. The aim of our trial was to study the relationships between climatic conditions and plant water status, to elucidate the different roles played by soil and air temperature. Olive plants were randomly divided in heated and non-heated soils and plant water status was weekly determined. In heated plants, soil temperature was maintained above 15 °C, while in non-heated soils, plants were maintained in field conditions. The effect of air temperature was determined by analyzing the plant's water status in heated plants, while the effect of soil temperature was studied through the plant water status differences between heated and non-heated soil treatments. Plant water status in the heated treatment fit into the vapour pressure deficit model, according to previous works conducted in warm conditions. Plant water status differences showed great variations in short periods of time, an unusual event with warm temperatures. In this study, two thresholds of soil temperature were found: above 10 °C, it had no effect on plant water status; between 10 °C and 6.5 °C, there was a linear relationship between plant water status and soil temperature, and below 6.5 °C, wind and relative humidity determined the plant water status. Our hypothesis is that low soil temperature produces a high resistance to water movement, while wind and relative humidity control the desiccation process in the leaf, so the great variations in plant water status are only at the leaf level and can therefore change quickly.

## 1. Introduction

The olive (*Olea europaea* L.) is a xerophytic evergreen tree that has been widely and traditionally cultivated in the Mediterranean region (Fernández-Escobar et al., 2012). This area is characterized by a mean annual temperature that ranges between 15–20 °C, with a minimum of 4 °C and a maximum of 40 °C, and an important soil water scarcity during the summer (Therios, 2009).

In the last 20 years, as a consequence of the many health benefits of the olive oil (Bernardiniand Visioli, 2017) and the increasing profitability of the olive farms, olive cultivation has experienced a significant expansion in valleys or higher latitudes, not only in the Mediterranean Region but also in new areas, where olive production is expanding and consumption is increasing. Thus, in the last few years, the highest relative increments in new olive plantations occurred in South America, South Africa, Australia and China (IOC, 2016). However, these new areas often experience low temperature conditions that can compromise olive production. Temperatures below -7 °C can reduce productivity by damaging the aerial parts of the plant, while temperatures below −12 °C are commonly associated with serious injuries that could threaten the life of the tree (Bongi and Palliotti, 1994).

Although olive trees are very sensitive to cold conditions, they begin an acclimation or hardening process in autumn in response to short day-lengths and low temperature conditions. During the hardening process, plants stop growing and undergo important metabolic changes (Bongi and Palliotti, 1994). This cold hardiness has been associated with a reduction in the starch reserves of the plant and an increase in their soluble sugar content (Drossopoulos and Niavis 1988; Eris et al., 2007). Such increases are believed to ameliorate the impact of the dehydration associated with freezing due to an increased level of solutes, which protect and support cellular structures under frost stress (Thomashow, 1999). In this sense, Cansev et al. (2009) and Gulen et al. (2009) studied the variations in tolerance to freezing in nine olive cultivars exposing olive leaves to low temperature at 4, −5, −10 and −20 °C. In the first work, authors found various antioxidative enzymes and dehydrin proteins associated with cold-acclimation or freezing tolerance in olive leaf tissues on a cultivar basis. In the second work, the authors concluded that soluble sugar and phospholipid compositions in

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olive leaves could have an important impact on the cold-acclimation process and on the susceptibility of the cell membranes to cold stress. Additionally, Arias et al. (2017) pointed out that during cold acclimation, a substantial water migration from apoplastic to symplastic compartments occurs in olive trees, enhancing their super-cooling capacity.

Different works have established that low temperature conditions cause leaf dehydration in sensitive plants (McWilliam et al., 1982; Aroca et al., 2001; Lee et al., 2005; Pérez-López et al., 2010, Arias et al., 2017). This chilling-induced dehydration is caused by an imbalance between water uptake by the roots and water lost through leaf transpiration as the result of an increase in soil-to-trunk hydraulic resistance (R) (Running and Reid, 1980; Pérez-López et al., 2010). Therefore, in chilling conditions, the regulation of root water uptake plays a more important role in overcoming stress injury than stomatal closure itself (Aroca et al., 2012). When soil temperature decreases below 15 °C olive trees behave similarly as if they were submitted to soil water deficit conditions, even if the soil water content is not limiting (Pérez-López et al., 2010). This behavior has also been observed in other crops (Kramer, 1940; McWilliam et al., 1982). In cold soil conditions, Kramer (1940) and Lyons and Raison (1970) observed a decrease in the membrane permeability of roots and an increase in the viscosity of water, which slowed its movement through both soil and roots. The reduction in the hydraulic conductivity of membranes by chilling may reduce the rate of efflux of water and potassium out of the guard cells, thus slowing the capacity of these cells to close in response to the decreasing water potential of the chilled leaf (McWilliam et al., 1982). The works conducted by García-Tejera et al. (2016) with potted plants indicated that the reduction of olive transpiration when root temperature decreased below a certain threshold could be due to an increase of the resistance from the soil to the root xylem, and more accurately to an increase of the resistance from the root surface to xylem vessels (Rp). In their work, Rp was related to the increase of water viscosity only when the temperature was above 15 °C; below this value, however, this variation could be explained by modifications in cell membrane structure, decreases in aquaporin amount and activity and/or suberin deposition in the root endodermis (Lee et al., 2005). In field conditions, López-Bernal et al. (2015) observed reductions in midday leaf water potential and stomatal conductance in the coldest period of winter despite evaporative demand being lower and soil water availability not being limiting. These reductions were associated with an increase in the soil-to-trunk hydraulic resistance and were dependent on the atmospheric conditions of the season, particularly precipitation, air temperature and vapour pressure deficit. However, the authors did not study the relationship between these parameters and the plant's water status.

The effects of chilling soil temperature on water relations of olive trees were also studied by Pavel and Fereres (1998) and Pérez-López et al. (2010). These authors, working with cv Picual and six different olive cultivars respectively, observed stem and leaf water potential reductions when soil temperature was lower than 10 °C. However, stomatal conductance was only reduced when temperature decreased below 6.4 °C in the results of the first work, while in the second one the threshold temperature at which this reduction was observed depended on the cultivar. These last responses were related to diverse chilling sensitivities due to differences in the response of their root water uptake rate to chilling stress (Aroca et al., 2001).

The aim of our work was to study the individual effects of low soil temperature and low air temperature conditions on olive water status. For this purpose, the relationships between the stem water potential and the meteorological conditions surrounding the plants were determined. These relationships could help with the understanding of how chilling weather conditions affect the plant's water status.

## 2. Materials and methods

The experiment started in the middle of October 2015 (day of the year, DOY, 295) and lasted until April 2016 (DOY 109). It was conducted at the Research Center "El Chaparrillo", part of the Regional Institute for Agro-Food and Forestry Research and Development (IRIAF from its initials in Spanish) located at Ciudad Real (Central Spain, 3° 56′W, 39° 0′ N, altitude 640 m). Eight olive trees cv. Arbequina, with a canopy diameter of around one meter and a shape similar to a sphere, which had fruit yields the previous year were selected. These plants had been grown in individual 0.030 $m^3$ pots. During the whole trial, all pots were irrigated daily until water drained in order to prevent lack of water.

The trees were randomly divided into two treatments, consisting of heated and non-heated soils. All the trees were transplanted into 0.044 $m^3$ pots before the experiment began. In the heated treatment, the transplanting allowed for the installation of a pipe around the root system. In this treatment, the pots were wrapped with expanded polystyrene to prevent heat exchange. A 30l tank was used to store the warm water. Inside it, a temperature probe (DS18B20 submersible probe, Dallas semiconductor, USA) was used to test the water temperature: when the temperature dropped below 18 °C, a heater inside the water tank turned on; if the water temperature still dropped further below 16 °C, a second heater turned on. When the water temperature increased until it reached these temperature thresholds, the heaters turned off automatically. In each heated pot, a temperature probe similar to the one inside the tank was installed 5 cm below the soil surface. If one of these soil temperature probes detected a temperature ≤16 °C, a water pump made water from the tank flow through the pipe installed around the root system in a closed circuit, afterwards returning the water to the tank (Fig. 1). The objective of this device was to maintain the soil temperature above 15 °C.

The other pots (non-heated soils) were installed one meter apart the heated pots in field conditions.

In both treatments, more accurate soil temperature probes (T-107, Campbell Sci, UK) were installed at a depth of 10 cm. Additionally, canopy temperature was measured in both treatments with temperature probes (T-107, Campbell Sci, UK) located inside the tree canopies. These temperatures were recorded every 5 min with a datalogger (CR-10X, Campbell Sci, UK).

Weekly throughout the trial, stem water potential (Ψs) was measured in each olive tree at midday. For this, fully expanded leaves were covered with aluminium foil for at least 45 min before removal; the water potential was measured with a pressure chamber (Soil Moisture Equip, Santa Barbara, Calif, USA). The stem water potential differential (ΔΨs), calculated as the difference between stem water potential obtained in the heated and non-heated treatments, was calculated in order to quantify the root cooling effect on plant water status.

Air temperature and relative humidity data were recorded every 30 min in a nearby (1 km away) meteorological station. Vapour Pressure Deficit (VPD) was calculated from these data.

The data were subjected to regression analysis using the Statistix 10 computer program. To check the regression model hypothesis (linearity, homoscedasticity, normality and independency), the Kolmogorov–Smirnov test was used with the Lilliefors correction, the Shapiro–Wilk tests for normality and the Levene's test for homoscedasticity on the identified residuals. For Levene's test, data were divided into four groups according to quartiles of the abscissa data. Linearity was observed in the graphics and independency was assumed due to the manner in which the data were obtained.

Midday stem water potential data were subjected to ANOVA, with the significance set at $P < 0.05$.

## 3. Results

The meteorological data were in agreement with historical climate data. During the experiment, the meteorological variables ranged as follow: temperature from −7.2 to 24.4 °C, relative humidity from 16.82% to 95.30%, daily average wind velocity from 0.26 to 6.84 $m\,s^{-1}$, and VPD from 0.06 to 0.58 KPa (Fig. 2).