



Daily whole-seedling transpiration determined by minilysimeters, allows the estimation of the water requirements of seedlings used for dryland afforestation



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ABSTRACT

In dryland areas, knowledge of plant water requirements and water use strategies of species are essential for use in afforestation, to ensure the establishment of seedlings in the first summer after planting. In this context, we used minilysimeters to estimate the daily whole-plant transpiration requirements in seedlings of *Pinus halepensis*, *Pistacia lentiscus* and *Quercus coccifera*, which are frequently used in afforestation. This estimate was calculated according to the following two conditions: high-soil moisture and a drought period. The results in both conditions indicated the highest daily rate ($0.90\text{--}1.33\text{ L m}^{-2}\text{ day}^{-1}$) for *Q. coccifera*, *P. lentiscus* was moderate ($0.50\text{--}0.75\text{ L m}^{-2}\text{ day}^{-1}$) and *P. halepensis* showed the lowest rate ($0.37\text{--}0.44\text{ L m}^{-2}\text{ day}^{-1}$). Species response under drought conditions was in accordance with their drought-avoidance strategy. *P. halepensis* and *P. lentiscus* displayed a water-saver mechanism, while *Q. coccifera* exhibited a water-spender mechanism. Our results show that the use of minilysimeters is a suitable method to determine whole-seedling transpiration rate and to accurately estimate the water requirements of species used for afforestation in dryland areas.

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1. Introduction

Among the main difficulties in afforestation in arid and semi-arid climates are high temperatures, low annual rainfall, vapour pressure deficit and low soil moisture (Vallejo et al., 2006; Vilagrosa et al., 2008). Evidence shows that after planting, seedlings suffer a post-transplant shock (Vilagrosa et al., 2001), as during their first summer they must withstand the climatic conditions mentioned above (Cortina et al., 2004). To overcome this critical time, efficiency in plant water supply under low soil moisture conditions can be fundamental in ensuring the establishment of seedlings in the first summer after planting (Mediavilla and Escudero, 2010; Verdaguer et al., 2011). In line with this, the adaptation strategy to drought (mainly in the seedling stage), is one

of the main physiological attributes considered in species selection, which allows plants to cope with severe drought periods (Cortina et al., 2004; Baquedano and Castillo, 2007).

Plant responses and plant water consumption under different drought conditions have been extensively studied (Vilagrosa et al., 2001; Baquedano and Castillo, 2006; Ramirez et al., 2007) and several methods have been used. Gas exchanges are often used to measure seedling water stand and transpiration (Alexou, 2013; Verdaguer et al., 2011; Vilagrosa et al., 2003b), and sap flow measures are often used in adult plants (Wallach et al., 2010; Molina and Del Campo, 2011; Chirino et al., 2011; Steppe et al., 2006). However, these measurements are usually obtained from leaves or small twigs, and whole seedlings are rarely measured. Therefore, in order to estimate the plant water consumption at an individual level (e.g. seedlings, saplings or trees), it is necessary to apply an up-scaling calculation method to calculate whole-plant consumption from an instantaneous transpiration rate, mainly when data are obtained by gas exchange (Lu et al., 2002; Vilagrosa et al., 2003b; Ramirez et al., 2008; Alvarez et al., 2011; Verdaguer et al., 2011; Chirino et al., 2011). Some up-scaling methods employ

Abbreviations: E_{w-s} , Whole-seedling transpiration rate; R_g , global radiation; E_{to} , reference evapotranspiration; VPD, vapour pressure deficit.

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morphological variables, e.g. the Leaf Area Index (Jonckheere et al., 2004; Weiss et al., 2004; Molina and del-Campo, 2011), while others employ above-ground biomass (Green et al., 2005; Navarro et al., 2006; Vazquez de Aldana et al., 2008; Castro and Freitas, 2009). In all cases, the risk of over or under estimation of plant water consumption is very high. Alternatively, gravimetric methods, e.g. load cells (mini- or minilymeters) or electronic balances can obtain more accurate measurements of the daily whole-seedling transpiration rate and the water consumption diurnal course (Rose et al., 1994; Ramirez et al., 2008; Mereu et al., 2009) as they allow the continuous monitoring and recording of weight variations in potted seedlings during different time intervals (hours and days) (Ramirez et al., 2008; Xin et al., 2008; Centinari et al., 2009; Müller and Bolte, 2009).

To avoid the estimation of daily transpiration rate by the up-scaling processes, we propose the use of minilymeters to determine the whole-seedling transpiration rate in three species commonly used in forest restoration programmes in semiarid areas of Spain: *Pinus halepensis* Miller, *Pistacia lentiscus* L and *Quercus coccifera* L. The main aim of this work was to determine whole-plant water consumption at an individual scale under contrasting wet-dry soil conditions, in continuous all-day records. The specific aims were to: (1) study the temporal dynamics of the daily whole-seedling transpiration rate under high and low soil moisture conditions; and (2) determine the relationship between the daily whole-seedling transpiration rate and soil moisture to assess drought tolerance limits before each species died.

2. Material and methods

2.1. Seedlings grown in pots

This study was carried out under full sunlight conditions in an experimental area of the University of Alicante. The (2-year-old) seedlings of *P. halepensis*, *P. lentiscus* and *Q. coccifera* were transplanted to pots (3.5 L) filled with forest soil substrate. This forest soil was analysed and characterized as clay loam with 2.95% of organic matter and 46.0% of total porosity. Before the experiment began, seedlings were grown for 5 months in full sunlight and under optimum soil moisture conditions to favour the growth of the new roots outside the root plug in order to colonise the soil in the pots. At the end of this nursery culture period, three potted seedlings per species were ready to be used to begin the experiments.

2.2. Monitoring the weight of potted seedlings with minilymeters

In order to estimate the transpiration rate of the potted seedlings and water loss by soil evaporation, minilymeters, constructed using load cells (models M-40, M-30, M-15, EPELSA, Spain) were used, which presented a maximum weight of 7 kg and a precision level of 0.001 kg. Using known weights within the 0–7 kg range, a calibration curve for each minilymeter was determined by linear equations, which allowed the signal to be converted from millivolts of the minilymeters into grams (Appendix A, Table A1). Next, each minilymeter was placed on a stable base in the experimental area and was connected to a CR800 datalogger (Campbell Scientific Ltd. UK) by means of a “multiplexer” (model AM16/32, Campbell Scientific Ltd., UK). The weight of each potted seedling was recorded hourly. The datalogger was scheduled with a “full bridge” algorithm using the PC400 Shortcut programme (Campbell Scientific Ltd. UK). For the purpose of knowing soil water evaporation loss, three pots were used without seedlings, which were filled with the same forest soil. These pots were not utilised as the control sample, but were taken as the reference soil water

evaporation value. Three potted seedlings per species and three pots filled with soil (without seedlings), each placed on a minilymeter, were all distributed according to a Latin square experimental design (Appendix B). The experiment set was protected on all sides by a 50-cm-high which avoided the effect of direct solar radiation on pots and of wind on the minilymeters. Finally, in order to avoid water loss through direct soil evaporation, a polyethylene bag was used to cover only the pots with seedlings, and a plastic flange was used to close the bag at the stem base.

2.3. The whole-seedling transpiration rate under high soil moisture conditions (experiment 1)

The transpiration rate was determined as the hourly and daily weight loss of potted seedlings, measured with minilymeters and recorded by taking the whole seedling as the sample unit. Therefore, the seedling transpiration rate is hereafter called the whole-seedling transpiration rate (E_{w-s}). The E_{w-s} under the high-soil moisture conditions was measured for 37 days (23 May to 29 June 2011). During the experiment, the potted seedlings were placed outdoors. In order to maintain the high-soil moisture conditions, each seedling was watered with 370 ml every 3 days. After watering, 12 h were allowed to elapse in order to obtain an adequate soil water distribution and this time interval was not considered valid to estimate E_{w-s} . Therefore, only the hourly weight measurements recorded on 3 consecutive days (0:00h–24:00 h solar hour) after watering were considered the valid sampling days. Rainy days were excluded.

The whole-seedling transpiration rate was analysed at two levels. On the one hand, the hourly whole-seedling transpiration rate was determined. For this purpose, the hourly difference in weight (g) of the potted seedlings measured by minilymeters was calculated and directly converted into litres of water (L). Then, hourly water loss (L) was normalised by the leaf area surface (m^2) of each seedling; which allowed the hourly whole-seedling transpiration rate (Hourly E_{w-s}) to be expressed as $L H_2O m^{-2} leaf area h^{-1}$. On the other hand, the daily whole-seedling transpiration rate was established as the difference in weight (g) of the potted seedlings, recorded every 24 h. This value was also normalised by the leaf area (m^2) of each seedling, and the daily whole-seedling transpiration rate (Daily E_{w-s}) was expressed as $LH_2O \cdot m^{-2} leaf area \cdot day^{-1}$.

2.4. The whole-seedling transpiration rate during a drought period (experiment 2)

To know the drought tolerance limits before each species died we followed one of the methods indicated by Pooter et al. (2009), which is suddenly withholding water after a life-long period of optimal supply with no acclimatisation period. The hourly and daily dynamics of E_{w-s} under water-deficit conditions was studied by means of a 25-day drought period (1–25 July 2011). Seedlings were watered to field capacity the day before the drought period began. Seedlings' drought response was monitored by measuring the hourly and daily E_{w-s} , and determined by the same methodology used to estimate both temporal levels during the experiment performed under high-soil moisture conditions. Low and constant E_{w-s} values indicated the end of the drought period in each species.

2.5. Microclimatic conditions and soil moisture

Microclimatic data and soil moisture were monitored during each experiment. The meteorological station (Campbell Scientific CR10 Ltd. UK) at the Climatology Laboratory of the University of Alicante, close to the experimental field site, monitored the continuous records of rainfall (mm), air temperature (T , °C), relative

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