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# Responses of Mediterranean ornamental shrubs to drought stress and recovery

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ABSTRACT

The aim of this study was to evaluate the differences in the mechanisms that are involved in the resistance of ornamental species to drought stress resulting from a regular suspension and recovery of the water supply. Plants of five ornamental shrubs [*Callistemon citrinus* (Curtis) Skeels (Callistemon), *Laurus nobilis* L. (Laurus), *Pittosporum tobira* (Thunb.) W.T. Aiton (Pittosporum), *Thunbergia erecta* (Benth.) Anderson (Thunbergia) and *Viburnum tinus* L. 'Lucidum' (Viburnum)] were subjected to two consecutive cycles of suspension/rewatering (S-R) and compared with plants that were watered daily (C). The relative water content (RWC), leaf water potential ($\Psi$), net photosynthetic rate ($A$), transpiration rate ($E$) and stomatal conductance ($Gs$) parameters were monitored during the experiment. The five species that were investigated exhibited different responses to drought stress. At the end of the experimental period, S-R treatment had no effect on dry weight in all species, except Pittosporum. In Pittosporum, drought stress reduced total plant biomass by 19%. Drought stress induced alterations in shrubs, including decreases in the shoot dry matter and increases in the root to shoot ratio, strongly affecting Callistemon and Pittosporum. All species adapted to water shortages using physiological mechanisms (RWC and water potential adjustment, stomatal closure and reductions in photosynthesis). Following rewatering, the species fully recovered and thus can be considered appropriate for green spaces in the Mediterranean environment. However, Laurus and Thunbergia seem to be less sensitive to drought stress than the other species.

## 1. Introduction

Water deficits are considered the main limiting factor for plant growth in the Mediterranean region during the summer because of the high levels of solar radiation and high temperatures (Di Castri, 1981). However, the global climate changes that this region has experienced during the last several decades have led to significantly increased water shortages throughout the year (IPCC, 2007; Wang et al., 2007). Thus, the issues that are linked to water shortage are of interest to landscape management, and the area of green sustainability is becoming increasingly relevant. The possibility of isolating specific plants that are resistant to particular abiotic stresses is currently being studied (Franco et al., 2006) because this knowledge is useful for the establishment of tools to improve sustainable green areas. The high number of ornamental plants that are used in Mediterranean areas (Romano, 2004) allows for the isolation of suitable genotypes that are able to cope with environmental stresses. All this has led to increased interest in the study of ornamental plant responses to water deficits in urban and suburban landscape environments.

Plant responses to drought are multiple and interconnected (Efeoğlu et al., 2009) and their capacities to adapt to this stress may vary considerably within genera and species (Sánchez-Blanco et al., 2002; Torrecillas et al., 2003). Mediterranean species have developed physiological and morphological adaptations to water stress (Dickson and Tomlinson, 1996), including the regulation of gas exchange (Moriana et al., 2002), osmotic adjustment (Chartzoulakis et al., 1999), the development of leaf protective structures (i.e., hairs, thick cuticles and schlerenchymatic cells), leaf modifications (i.e., inclination variations, increased thicknesses and reduced surface areas) (Castro-Díez et al., 1998; Gratani and Bombelli, 2000; Karabourniotis, 1998) and more extensive root systems (Malinowski and Belesky, 2000). Numerous morphological adaptations to water stress involve the aerial portions of plants. Leaf growth is the most sensitive plant process to water deficits

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*Abbreviations:* $A$, net photosynthetic rate; ANOVA, analysis of variance; DW, dry weight; $E$, transpiration rate; $G_s$, stomatal conductance; RWC, relative water content; S-R, cycle suspension/rewatering; SLA, specific leaf area; R/S, root/shoot ratio.

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(Bradford and Hsiao, 1982; Hsiao, 1973; Jones, 1985). The vertical orientations of the leaves allow the plants to reduce the radiant energy that is intercepted (Pereira and Chaves, 1993). The specific leaf area, which is often used as an indirect indicator of leaf thickness, is reported to be reduced under drought conditions (Liu and Stützel, 2004; Marcelis et al., 1998). The reduction of the specific leaf area is assumed to be a way to improve water use efficiency (WUE) (Craufurd et al., 1999; Wright et al., 1994) because thicker leaves usually have higher densities of chlorophyll and proteins per unit leaf area and thus have greater photosynthetic capacities per unit leaf area than thinner leaves (Liu and Stützel, 2004). The specific leaf area was shown to be reduced in *Asteriscus maritimus* following water stress as a direct consequence of reduced leaf areas (Rodríguez et al., 2005). Similar results were found for *Eragrostis curvula*, *Oryza sativa*, *Abelmoschus esculentus* and *Asteriscus maritimus* following water stress, and significantly decreased total leaf areas were observed (Rucker et al., 1995; Shubhra et al., 2003).

Several authors have found frequent increases in the root-shoot ratios in plants under water stress (Blum, 1996; Zwack and Graves, 1998), which has been considered to be an adaptive strategy (Bargali and Tewari, 2004; Guo et al., 2007; Li et al., 2008) because a larger investment in roots improves the absorption of water.

Reduced photosynthesis is one of the main consequences of water stress (Hsiao and Acevedo, 1974; Huang, 2004) and is related to stomatal closure, which is implemented by the plant to reduce water loss through transpiration (Nayyar and Gupta, 2006; Yang et al., 2006). However, the duration and speed of the stomatal closure vary depending upon the species (Schulze and Hall, 1982).

Evergreen trees have adopted mechanisms to cope with the typical conditions of the Mediterranean, including the ability to endure water limitation and to recover after rainfall (Galmés et al., 2007). Further, lemon plants respond to water stress and rewatering by developing drought avoidance mechanisms, such as stomatal closure, leaf rolling and partial defoliation (Ruiz-Sànchez et al., 1997). Efeoğlu et al. (2009) demonstrated that the relative water content in maize was significantly reduced under drought stress conditions but significantly increased during the recovery period, reaching the levels of the control plants. Other authors (Sánchez-Blanco et al., 2002) have shown that plants of *Cistus albidus* and *C. monspeliensis* that experienced water stress and recovery have developed different avoidance mechanisms, for example, *C. albidus* limits growth and cell expansion, while *C. monspeliensis* reduces photosynthetic processes.

Water stress could limit plant vegetative growth, performance and also the survival of shrubs and trees (Fernández et al., 2006), and consequently, the selection of drought-tolerant plants may be considered a strategy for the improvement of landscape management (Niu et al., 2008). However, information regarding the responses of some ornamental species in Mediterranean environments to short-term water stress is still lacking. Thus, the aim of this study was to evaluate differences in the mechanisms that are involved in the resistance of ornamental species to water stress as a result of a regular suspension and recovery of the water supply. These different mechanisms were studied in five ornamental shrubs that are commonly used in Mediterranean landscapes.

## 2. Material and methods

### 2.1. Plant materials, growing conditions and experimental treatments

The experimental trial was carried out in an unheated greenhouse that was located in Catania, Italy (37°30′N 15°06′E 20 m a.s.l.). The five ornamental shrubs [*Callistemon citrinus* (Curtis) Skeels (Callistemon), *Laurus nobilis* L. (Laurus), *Pittosporum tobira* (Thunb.) W.T. Aiton (Pittosporum), *Thunbergia erecta* (Benth.) Anderson (Thunbergia) and *Viburnum tinus* L. 'Lucidum' (Viburnum)], from commercial nursery, were transplanted into 3.3-L pots (one plant per pot) that were filled with a mixture of sand (75%), silt (18%) and clay (7%). Six-month-old plants were watered daily to pot capacity (determinated by gravimetric method) at regular intervals prior to the initiation of the treatments using a drip irrigation. The initial biomasses of the plants were 69.3 g plant$^{-1}$ (Callistemon), 180.6 g plant$^{-1}$ (Laurus), 172.6 g plant$^{-1}$ (Pittosporum), 145.6 g plant$^{-1}$ (Thunbergia) and 38.4 g plant$^{-1}$ (Viburnum). After 30 days, 30 plants from each species were subjected to two consecutive cycles of suspension/rewatering (S-R), while another 30 plants from each species were watered daily (C). For every cycle in the S-R treatment, the water suspension lasted for 7 days, after which the plants were rewatered to pot capacity for another 7 days. After the second water suspension cycle, S-R treatment plants were maintained under the same conditions as the control plants for 14 days (Fig. 1).

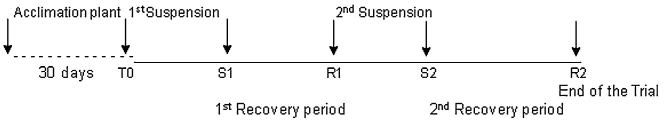

**Fig. 1.** Timeline for suspension/rewatering (S-R) treatments used in experiment. Plants were irrigated daily to container capacity or subjected to suspension/rewatering treatment [Start trial (T0), 7 days no water (S1), 7 days daily irrigation (R1), 7 days no water (S2), 14 days daily irrigation (R2)].

The mean air temperatures, relative humidity levels and global radiation levels during the experimental periods were recorded on a data logger (CR 1000; Campbell Scientific Ltd., Loughborough, UK). The maximum and minimum temperatures were 22.6 and 18.2 °C, respectively, and the mean relative humidity levels ranged from 60 to 68%. The total radiation levels ranged from 8.2 to 11.6 MJ m$^{-2}$.

### 2.2. Data collection

On days 37 (S1), 44 (R1), 51 (S2) and 64 (R2) (Fig. 1) of the experimental period, the midday relative water content (RWC) and the midday leaf water potential ($\Psi$) were measured between 12:00 and 14:00 (solar time). The RWC were measured on fully opened leaves. Five leaf discs that were 10 mm in diameter were excised from the interveinal areas of each plant. For each replicate, 30 discs were pooled, and their fresh weights (FW) were determined. They were floated on distilled water in Petri dishes for 4 h to regain turgidity and then the turgid tissue was quickly blotted to remove excess water and reweighed [turgid weight (TW)]. The samples were dried at 80 °C for 24 h to determine the dry weights (DW) (Rouphael et al., 2008). The RWC were calculated according to Jones and Turner (1978):

$$\text{RWC\%} = (\text{FW} - \text{DW}/\text{TW} - \text{DW}) * 100$$

The leaf water potential were estimated according to Scholander et al. (1965) using a pressure chamber (PMS Model 1000, Instrument Company, Albany, Oregon, USA). The leaves were removed with a sharp blade near the petiole bases and immediately tested.

On days 30 (T0), 37 (S1), 44 (R1), 51 (S2) and 64 (R2) (Fig. 1) of the experimental period, the net photosynthetic rate (*A*), transpiration rate (*E*) and stomatal conductance (*Gs*) were measured on mature, fully expanded leaves using a $CO_2/H_2O$ IRGA (LCi, ADC Bioscientific Ltd., Hoddesdon, UK). The measurements were carried out in clear conditions from 10:00 to 13:00 (solar time). All of the photosynthesis measurements were performed on outer, fully expanded leaves that were sampled from branches that were located in the middle of the canopy.

At the end of the experiment, nine pots per treatment (three per replication) were randomly chosen for the measurement of