

Changes in tissue-water relations, photosynthetic activity and growth of *Myrtus communis* plants in response to different conditions of water availability

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SUMMARY

The influence of different levels of water deficit on physiological and morphological alterations in *Myrtus communis* plants was investigated to evaluate their adaptability to such conditions. *M. communis* plants growing under greenhouse conditions were subjected to three irrigation treatments between February and August 2007: a control, and two water deficit treatments. Plants submitted to severe water deficit showed reduced shoot and root dry weights, leaf numbers, leaf areas and plant heights, while moderate water deficit reduced only plant height. Leaf colour was not affected by either water deficit treatment. Root hydraulic resistance increased proportionally to the level of drought and lower values of leaf water potential at pre-dawn were observed in both deficit treatments. The absence of osmotic adjustment could explain the reduction of leaf turgor potential at midday. Photosynthesis decreased in both water deficit treatments and was related to stomatal factors, since no significant changes in the values of chlorophyll fluorescence, chlorophyll content or ion leakage were observed. The highest leaf water potential values (Ψ_l) were found in the early morning and the lowest at midday, in all treatments; the latter coincided with the minimum values of stomatal conductance. Significant differences in Ψ_l values during the day were noted between treatments, but were always highest in the control. Cell wall rigidity, measured as increased bulk modulus of elasticity increased under severe water stress resulting in a loss of turgor at lower leaf water potential values.

Evergreen shrub species in the Mediterranean area may adapt morphologically and physiologically to protect against summer drought (Krause and Kummerow, 1977; Miller and Poole, 1979). In spite of this, these shrubs may be affected by the decrease in the available of soil water or even suffer considerable water stress during the summer dry season (Tenhunen *et.al.*, 1985; Lo Gullo and Salleo, 1988; Rhizopoulou and Mitrakos, 1990), since only some of the changes observed in the plants under drought may confer stress-resistance to the plant (Hsiao *et al.*, 1976). In general, plant responses depend on the severity and duration of the stress, species, development stage and interactions with environmental factors (Rhizopoulou and Mitrakos, 1990; Bowman and Roberts, 1985). Such a complexity of factors makes it difficult to describe which responses increase resistance.

Low water availability is considered the main environmental factor limiting photosynthesis, and consequently plant growth (Flexas *et al.*, 2004). Also, tissue water relations and gas exchange vary in response to changes in water availability (Rhizopoulou and Mitrakos, 1990; Tognetti *et al.*, 2000b). However, these changes may imply different water relations and morphological strategies. Stomatal conductance closure, osmotic adjustment, changes in cell wall elasticity and reductions in aerial part growth (Tyree and Jarvis, 1982; Zollinger *et al.*, 2006) may improve plant water status and the resistance of plants to water stress, limiting the loss water under high evaporative demand (Sánchez-Blanco *et al.*, 2004).

Myrtus communis L. is a sclerophyllus evergreen shrub (Mendes *et al.*, 2001) of interest for ornamental use in revegetation projects in semi-arid degraded land and in landscaping (Romani *et al.*, 2004). Although, *M. communis* is a typical Mediterranean species with good adaptability to environmental stresses, it may, under natural conditions, suffer drought stress associated to high solar radiation (Gucci *et al.*, 1998; Mendes *et al.*, 2001). Little is known about its physiological responses to different degrees of drought. The purpose of this work was to study both diurnal and seasonal physiological patterns in *M. communis*

plants exposed to different levels of water deficit to measure changes in the growth, ornamental characteristics, water relations, gas exchange, and photosynthetic efficiency developed by this species to help it adapt to drought stress situations.

MATERIALS AND METHODS

Plant material and experimental conditions

Seedlings (150) of 2-year-old native myrtle (*Myrtus communis* L) were grown in 3.6 l plastic pots filled with a 2:1:1 (v/v/v) mixture of coconut fibre:black peat:perlite, amended with 2 g l⁻¹ substrate of Osmocote Plus (14:13:13 N,P,K plus microelements). The experiment was conducted in 2007 at Santomera (Murcia, Spain) in a plastic greenhouse equipped with a cooling system. The micro-climatic conditions, registered with an Escort Junior Data Logger (Escort Data Loggers, Inc., Buchanan, Virginia, USA) were 4°C (minimum), 38°C (maximum), and 25°C (average) temperatures; and 21% (minimum), 100% (maximum) and 60% (average) relative humidities.

Treatments

After 2 months in the greenhouse, the plants were subjected to three irrigation treatments (50 plants per treatment) irrigated using a computer-controlled drip irrigation system from February to August 2007. The irrigation treatments consisted of 100% water holding capacity [(leaching 15% (v/v) of the applied water; Control)], 60% of the control irrigation water (moderate water deficit; MWD), and 40% of the control irrigation water (severe water deficit; SWD). One drip nozzle delivering 2 l h⁻¹ per pot was connected to two spaghetti tubes (one each side of every pot) and the duration of each irrigation episode was used to vary the amount of water applied, which depended on the season and on climatic conditions. The volume of water varied between 400 and 700 ml per pot for the controls, and

the irrigation frequency was set to maintain the soil matric potential (SMP) between -20 kPa and -40 kPa. The SMP was registered using six watermark probes (Termistor 107; Campbell Scientific S.L., Barcelona, Spain).

Growth and ornamental measurements

At the end of the experimental period, all substrate was gently washed from the roots of ten plants per treatment and the plants were divided into shoots (i.e., leaves and stems) and roots. These were then oven-dried at 80°C until they reached a constant weight to measure the respective dry weight (DW). Plant heights (cm), leaf numbers and leaf areas (cm²) using a leaf area meter (Delta-T; Devices Ltd., Cambridge, UK), were determined in the same plants.

Leaf colour was measured with a Minolta CR-10 colorimeter (Konica Minolta Sensing Inc., Osaka, Japan), which provided the colour coordinates of hue angle (h°), chroma (C^*) and lightness (L^*) (McGuire, 1992). Three leaves were measured on each plant, and ten plants were studied per treatment. The relative chlorophyll content (RCC) was measured using a Minolta SPAD-502 chlorophyll meter (Konica Minolta Sensing Inc., Osaka, Japan) at the midpoint of each mature leaf using the same leaves as were used for the colorimetric measurements.

Physiological measurements

Water relations and gas exchange: Seasonal changes in leaf water potential (Ψ_l), leaf osmotic potential (Ψ_s) and leaf turgor potential (Ψ_t) at dawn and at midday, leaf osmotic potential at full turgor (Ψ_{100s}), stomatal conductance (g_s), and net photosynthesis (P_n) at midday, were measured on five plants per treatment. At the end of the experimental period the diurnal patterns of Ψ_b , Ψ_s , Ψ_t , g_s , and P_n were measured from sunrise to sunset, at 2 h intervals, also in five plants per treatment.

Leaf water potential was estimated according to Scholander *et al.* (1965), using a pressure chamber (Model 3000; Soil Moisture Equipment Co., Santa Barbara, CA, USA) in which leaves were placed in the chamber within 20 s of collection and pressurised at a rate of 0.02 MPa s⁻¹ (Turner, 1988). Leaves from the Ψ_l measurements were frozen in liquid nitrogen (-196°C) and stored at -30°C. After thawing, the osmotic potential (Ψ_s) was measured in the extracted sap using a WESCOR 5520 vapour pressure osmometer (Wescor Inc., Logan, UT, USA), according to Gucci *et al.* (1991). Ψ_t was estimated as the difference between leaf water potential (Ψ_l) and leaf osmotic potential (Ψ_s). Leaf osmotic potential at full turgor (Ψ_{100s}) was estimated as indicated above for Ψ_s , using excised leaves with their petioles placed in distilled water overnight to reach full saturation.

Leaf stomatal conductance (g_s) and net photosynthetic rate (P_n) were determined on attached leaves using a gas exchange system (LI-6400; LI-COR Inc., Lincoln, NE, USA). Chlorophyll fluorescence measurements were taken in five plants per treatment at midday, on the adaxial leaf surface. Water deficit and control leaves were re-darkened for 20 min before starting the measurements (Camejo *et al.*, 2005). Initial fluorescence (F_o) was measured using a weak, modulated red light. Maximum chlorophyll fluorescence (F_m) was measured after a 0.8⁻¹s pulse of strong red light (>4000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ PAR). The values of F_o , F_m and maximum photochemical efficiency of photosystem II (F_{vm}) were obtained using a portable fluorometer Opti-Sciences (Model OS 30; Opti-Sciences Inc., Tyngsboro, MA, USA).

Pressure-volume curves: Estimates of the relative water content at the turgor loss point (RWC_{tlp}), the leaf water potential at the turgor loss point (Ψ_{tlp}) and the bulk modulus of elasticity (ϵ), were obtained at the end of the experimental period using three leaves per plant and five plants per treatment. Pressure-volume analysis of the leaves was performed as

outlined by Wilson *et al.* (1979). The bulk modulus of elasticity (ϵ) at 100% *RWC* was calculated using the formula:

$$\epsilon = (RWC_{tlp} \times \Psi_{100s}) / (100 - RWC_{tlp})$$

where ϵ is expressed in MPa, Ψ_{100s} is the osmotic potential at full turgor (MPa), and RWC_{tlp} is the relative water content at the turgor loss point expressed as a percentage.

Leaves were excised in the dark, placed in plastic bags, and allowed to reach full turgor by dipping the petioles in distilled water overnight (Davis and Mooney, 1986). Pressure-volume curves were obtained from periodic measurements of leaf weight and balance pressure as the leaves dried on the bench at a constant temperature of 20°C. Drying-leaves period in each curve was approx. 4 – 5 h.

Hydraulic resistance: Hydraulic resistance ($1/L_p$) was determined at the end of the experimental period in five plants per treatment as the inverse of the root hydraulic conductivity (L_p), measured according to Ramos and Kaufmann (1979).

Plants were de-topped and the substrate was carefully washed from the roots, which were submerged in a container of water and placed in the pressure chamber with the cut stump exposed. The air pressure was increased in the chamber at an approx. rate of 0.4 MPa min^{-1} , up to a final pressure of 0.8 MPa. A small piece of plastic tubing was fitted to the stump and, every 5 min, the exudate was collected and its volume measured. After the exudation measurements, the root systems were placed in an oven at 80°C until they reached a constant DW. Root hydraulic conductivity was calculated using the formula:

$$L_p = J / (P \times W)$$

where L_p is expressed in $\text{mg g}^{-1} \text{s}^{-1} \text{MPa}^{-1}$, P is the applied hydrostatic pressure (MPa), W is the DW of the root system (g), and J is the water flow rate through the entire root system (mg s^{-1}).

Ion leakage: The rates of passive leakage from sensitive tissue are used as a measure of alterations in membrane permeability. In our case, ion leakage was estimated according to the method described by Lafuente *et al.* (1991).

Thirty leaf discs, 2 mm in diameter, from each plant and eight replicates per treatment were incubated in 10 ml 0.3 M mannitol in 50 ml centrifuge tubes. The tubes were shaken at 120 cycles min^{-1} and the conductivity of the solution was measured after 24 h with a Crison Model 524 digital conductivity meter (Crison Instruments, S.A. Barcelona, Spain). Tubes containing the solution were weighed and heated to boiling for 10 min. After cooling to room temperature, while still shaking, deionised water was added to restore their initial weight and the total conductivity was measured after an additional 0.5 h of shaking. Ion leakage rates were expressed as the percentage of the total conductivity.

Statistical analyses

The data were analysed by one-way ANOVA using Statgraphics Plus for Windows 5.1 Software. Treatment means were separated with Duncan's Multiple Range Test ($P \leq 0.05$).

RESULTS

At the end of the experimental period, the severe water deficit (SWD) treatment was seen to have reduced shoot and root DWs, the numbers of leaves, leaf areas and the root/shoot ratios of *M. communis* plants compared with the controls and those exposed to moderate water deficit (MWD; Table I). Plant height was significantly inhibited by both water deficit treatments, with reductions of 9.3% and 15.6% for MWD and SWD, respectively. Leaf colour parameters (L^* , C^* and h°), relative chlorophyll contents (RCC) and ion leakage values were not affected by the water conditions of the substrate (Table I).

The water deficit treatments caused significant differences in the water relations of the myrtle plants (Table II; Figure 1-3). The water deficits applied produced increases in root hydraulic resistance proportional to the level of drought imposed, with values of 1.4, 3.3, and 5.4 g MPa s mg⁻¹ recorded for the controls, MWD, and SWD treatments, respectively (Table II). This could affect to the seasonal values of leaf water potential (Ψ_l) at pre-dawn [(from -0.25 to -0.44 MPa for the controls, from -0.29 to -0.53 MPa for MWD, and from -0.37 to -0.54 MPa for the SWD (Figure 1B)]. However, although of the highest values for root hydraulic resistance were observed in SWD, the plants in this treatment had similar Ψ_l values to the MWD plants. The differences in Ψ_l values at midday between treatments were lower than at predawn due to the influence of environmental factors (Figure 1B). The Ψ_l values decreased in all treatments as the evaporative demand of the atmosphere increased (July and August), leading to lower leaf turgor potential (Ψ_l) values at midday (Figure 1A). No differences in Ψ_{100s} between treatments were found during the experimental period (Figure 2), pointing to an absence of osmotic adjustment, which might explain the reduction observed in Ψ_l values at midday.

Parameters derived from the pressure-volume curves are shown in Table II. The water potential at turgor loss point (Ψ_{tlp}) was affected by the severe water deficit, showing values of -3.11 MPa (Table II). The bulk modulus of elasticity (ϵ) increased in the SWD treatment (Table II).

The highest values of g_s and P_n corresponded to the control plants. Both g_s and P_n decreased similarly in both water deficit treatments (Figures 1C, D), as did Ψ_l (Figure 1B). No changes were observed in the chlorophyll fluorescence (F_{vm}) values, which remained at around 0.8 in all treatments (Table I).

At the end of the experimental period (August), the highest Ψ_l values were found early in the morning and the lowest at midday (Figure 3B), coinciding with the minimum g_s levels

(Figure 3D), after which, the Ψ_l and Ψ_t values recovered. Significant differences in Ψ_l levels were noted between treatments, although they were always higher in the control than in either water deficit treatment.

DISCUSSION

Growth reduction as a result of water deficit has been widely reported in different ornamental species (Sánchez-Blanco *et al.*, 2002; Franco *et al.*, 2006). However, the intensity of the plant response can vary, depending on the stress level and duration (Cameron *et al.*, 1999). The water deficit stress levels applied in our assay led to substantial differences in the growth of myrtle plants, while moderate water stress produced no significant changes in plant development (with the exception of plant height), a greater water deficit clearly reduced all plant growth parameters (Table I). This finding may be important for grower of ornamental plants because plants are often exposed to drought treatments during nursery production to reduce excessive growth. However, it goes without saying that it is first necessary to know the level of drought to which a species to maintain healthy growth and acceptable quality (Henson *et al.*, 2006).

The distribution of assimilates from the aerial part to the root system in water stress situations has been observed by several authors in different species, such as *Rosmarinus officinalis* (Sánchez-Blanco *et al.*, 2004), *Lotus creticus* (Bañón *et al.*, 2004), *Limonium cossonianum* (Franco *et al.*, 2002) and *Argyranthemum coronopifolium* (De Herralde *et al.*, 1998). In our conditions, the root/shoot ratio did not increase in the *M. communis* plants exposed to water deficit (Table I). Although this response may be more useful in field conditions, the morphological characteristics of plant growth in nursery conditions (in potted plant) may be the determining factors for subsequent establishment and survival after transplantation for landscaping and gardening purposes (Kailash and Kannan, 1999). No

significant degree of leaf abscission was observed, but both leaf numbers and leaf areas decreased as a result of the severe water deficit, which could reflect a drought avoidance mechanism to reduce water loss through the leaves, thus contributing to the water economy of the plant (De Herralde *et al.*, 1998; Bañón *et al.*, 2002). However, the leaf colour was not modified by the water deficit treatments, suggesting that plants can cope with water shortage without losing their ornamental value (Sánchez-Blanco *et al.*, 2009).

Increased water flow resistance from the substratum to the plant in water stress conditions has been observed in numerous species (Sánchez-Blanco *et al.*, 2002; De Herralde *et al.*, 1998) and, in our case, this phenomenon could have minimised water transport toward the leaves. Such a response would help explain the lowest leaf water and leaf turgor potential values in water deficit treatments, especially at dawn (Figures 1A, B). Thus, the absence of osmotic adjustment (Figure 2) could explain the turgor loss at midday. Several factors influence the existence of an osmotic adjustment, including stress intensity and the species (Cutler *et al.*, 1980; Turner and Jones, 1980). In our experiment, the Ψ_{100s} values were similar and independent of the level of water stress applied. According to Tognetti *et al.* (2000c), many Mediterranean shrubs showed a small degree of active osmotic adjustment or/and adjustments of little importance in drought resistance. As reported for other Mediterranean shrubs (Davis and Mooney, 1986), turgor potentials were not constant throughout the day or during the whole study period (Figures 1A and 3A). Minimum values (never below 0) were reached in mid-summer and maximum values occurred at predawn, both similar to those found in *M. communis* plants by Tognetti *et al.* (2000c).

Increased rigidity of the cell walls (increased bulk modulus of elasticity) in the *M. communis* plants exposed to severe water stress was accompanied by lower Ψ_{tlp} values, which indicates that the turgor loss point was reached at lower leaf water potential. These values coincided with those reported by Tognetti *et al.* (2000c) in this same species. Also, a small

cell volume and a large apoplastic water fraction have generally been associated with decreased cell tissue elasticity (Cutler and Rains, 1978) which appears to be a typical characteristic of sclerophyllous habits.

The ability to control water loss (g_s reduction) is another important mechanism for reducing water loss through transpiration. Stomatal opening may promote a decrease in photosynthetic activity (Flexas *et al.*, 2004). In our experiment, the lower P_n observed in the stressed plants depended on stomatal factors, since no significant changes were observed in the F_{vm} values (Table I), indicating the lack of drought-induced damage of the photochemistry PSII in *M. communis* plants, as has been reported for many species (Cornic, 1994; Munné-Bosch *et al.*, 2009). According to Corlett and Choudhary (1993), the photochemical efficiency measured as F_{vm} is only affected when the water stress in horticultural species is very severe. In this sense, Gallé *et al.* (2007) reported that *Quercus pubescens* seedlings reduced photosynthetic activity, mainly as a result of decreased stomatal conductance, to protect themselves against water loss and dehydration in drought situations. This decrease in photosynthesis could affect plant growth, although the differences observed in P_n were not so evident as those observed for the growth parameters in both water deficit plants. No changes in the ion leakage values were observed (Table I), suggesting that membranes were undamaged.

Seasonal and diurnal patterns of stomatal conductance in Mediterranean sclerophyllous species exhibit a pronounced morning peak followed by partial closure, afterwards coinciding with decreasing leaf water potential (Rhizopoulou and Mitrakos, 1990; Gill and Mahall, 1986). This process was also observed in myrtle plants and would represent a response to limit water losses via transpiration and to optimize the use of water resources at moments of higher evaporative demand (Tenhunen *et al.*, 1990). This behaviour was reflected in the Ψ_l values of water deficit plants, since much lower values were not reached. In previous

studies in Mediterranean species, gas exchange was more closely related to leaf water potential than to turgor pressure (Serrano and Peñuelas, 2005). Also, in some Mediterranean shrubs, including *M. communis* it has been observed that stomata regulate leaf water status in coordination with soil water potential and hydraulic resistance (Tognetti *et al.*, 2000b).

In conclusion the level of drought to which *M. communis* plants were exposed led to significant differences in growth, although, such growth was not always related with the plant water status (e.g. leaf water potential and turgor pressure). The mechanisms used by this species to protect against drought were mainly based on responses to avoid water losses through transpiration, e.g. decreased stomatal conductance and, in the case of more severe water stress, reductions in leaf area and leaf number. The water deficit response observed in this study suggests that *M. communis* is well-adapted to withstand water stress periods that are frequent in Mediterranean ecosystems.

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TABLE I

*Influence of irrigation treatments on growth, colour parameters, relative chlorophyll content (RCC), chlorophyll fluorescence (F_{vm}) and ion leakage in *M. communis* plants at the end of the experiment*

Parameter	Control	MWD (60%)	SWD (40%)
Plant height (cm)	28.49 b	25.84 a	24.04 a
Shoot dry weight (g plant ⁻¹)	14.04 b	12.38 b	8.99 a
Root dry weight (g plant ⁻¹)	11.96 b	10.95 b	6.79 a
Root/shoot ratio	0.861 b	0.893 b	0.761 a
Leaf number	810.3 b	800.1 b	533.1 a
Leaf area (cm ²)	684.1 b	593.2 b	446.6 a
Lightness (L^*)	48.14 a	50.46 a	51.43 a
Chroma (C^*)	31.69 a	34.94 a	33.69 a
Hue angle (h°)	111.75 a	115.84 a	106.26 a
RCC	36.35 a	30.06 a	32.75 a
F_{vm}	0.796 a	0.778 a	0.759 a
Ion leakage (%)	31.35 a	33.58 a	35.16 a

Means values in each row followed by a different lower-case letter are significantly different by Duncan

MRT at $P \leq 0.05$.

TABLE II

*Influence of irrigation treatments on leaf water relations parameters derived from pressure-volume curves and root hydraulic resistance ($1/L_p$) in *M. communis* plants at the end of the experiment*

Parameter	Control	MWD (60%)	SWD (40%)
Ψ_{tp} (MPa)	-2.62 a	-2.65 a	-3.11 b
RWC_{tp} (%)	77.17 a	74.51 a	72.84 a
ε (MPa)	6.86 a	8.01 ab	9.22 b
$1/L_p$ ($\text{mg g}^{-1} \text{s}^{-1} \text{MPa}^{-1}$)	1.434 a	3.322 b	5.440 c

Means values in each row followed by a different lower-case letter are significantly different by Duncan

MRT at $P \leq 0.05$.

FIG.1

Seasonal patterns of leaf turgor potential at pre-dawn (pd) and midday (md) (Ψ_t ; Panel A), leaf water potential at pre-dawn and midday (Ψ_l ; Panel B), net photosynthesis (P_n ; Panel C), and stomatal conductance (g_s ; Panel D) at midday in *M. communis* plants under different irrigation treatments: Control, MWD (moderate water deficit) or SWD (severe water deficit). Values are means ($n = 5$) and vertical bars indicate \pm SE.

FIG.2

Seasonal pattern of leaf osmotic potential at full turgor at midday (Ψ_{100s}) in *M. communis* plants under different irrigation treatments: Control, MWD (moderate water deficit) or SWD (severe water deficit). Values are means ($n = 5$) and vertical bars indicate \pm SE.

FIG.3

Diurnal time-courses of leaf turgor potential (Ψ_t ; Panel A), leaf water potential (Ψ_l ; Panel B), net photosynthesis (P_n ; Panel C) and stomatal conductance (g_s ; Panel D) at the end of the experimental period in *M. communis* plants under different irrigation treatments: Control, MWD (moderate water deficit) or SWD (severe water deficit). Values are means ($n = 5$) and vertical bars indicate \pm SE.

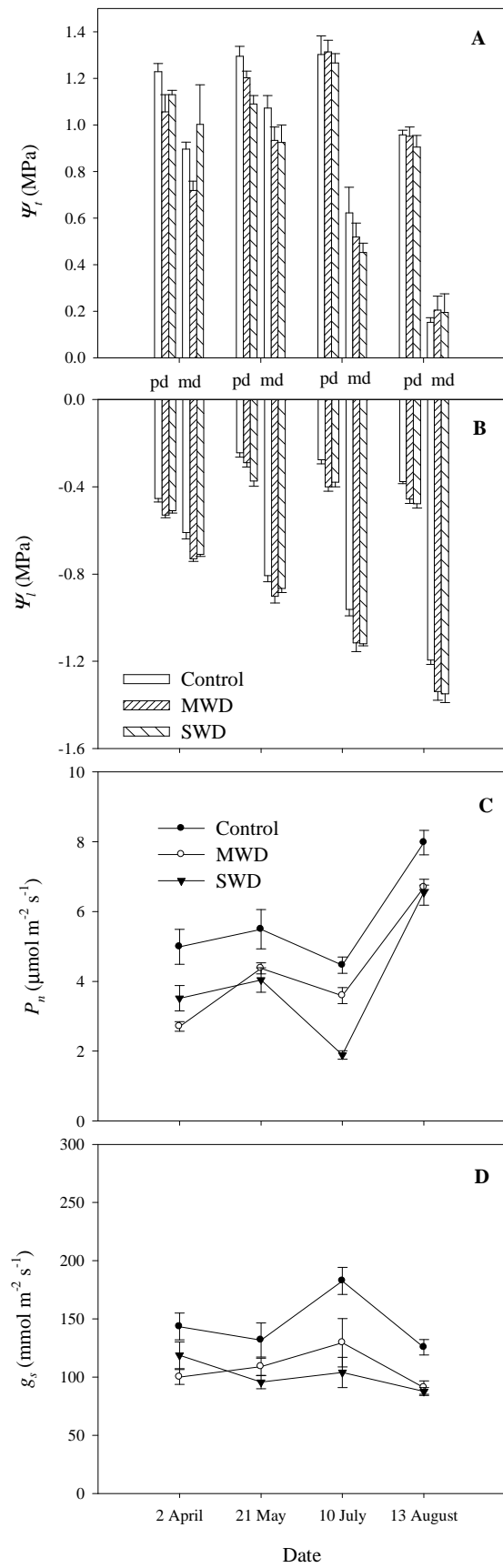


FIG. 1, NAVARRO, A.

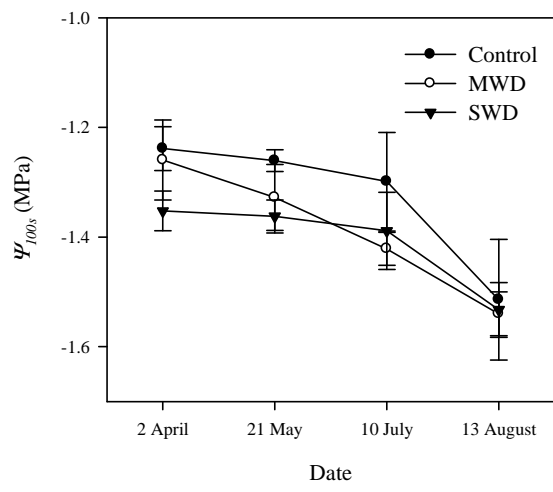


FIG.2, NAVARRO, A.

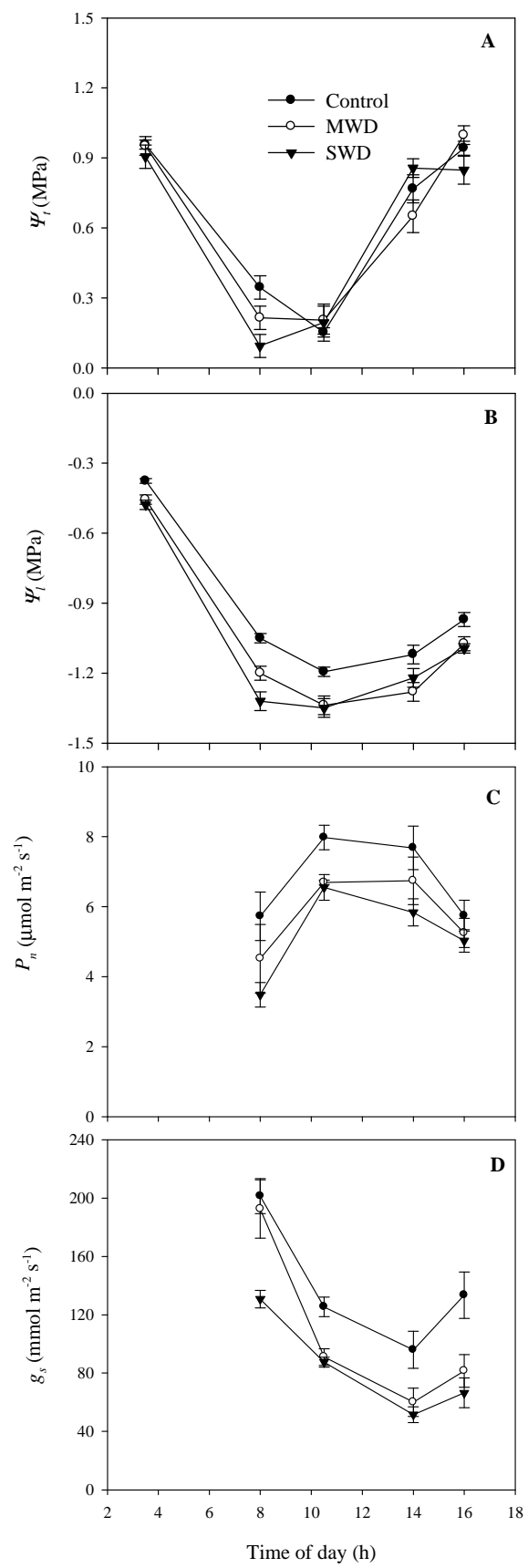


FIG. 3, NAVARRO, A.