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Effects of water stress and rewatering on leaf water relations of lemon plants

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Abstract

Potted two-year-old lemon plants (*Citrus limon* (L.) Burm. fil.) cv. Fino, growing under field conditions were subjected to drought by withholding irrigation for 13 d. After that, plants were re-irrigated and the recovery was studied for 5 d. Control plants were daily irrigated maintaining the soil matric potential at about -30 kPa. Young leaves of control plants presented higher leaf conductance (g_l) and lower midday leaf water potential (Ψ_{md}) than mature ones. Young leaves also showed higher leaf water potential at the turgor loss point (Ψ_{tlp}) than mature leaves. In both leaf types g_l decreased with increased vapour pressure deficit of the atmosphere. From day 1 of the withholding water, predawn and midday leaf water potentials (Ψ_{pd} and Ψ_{md}) decreased, reaching in both cases minimum values of -5.5 MPa, with no significant differences between mature and young leaves. Water stress induced stomatal closure, leaf rolling and partial defoliation. No osmotic adjustment was found in response to water stress in either leaf type, but both were able to enhance the cell wall elasticity (elastic adjustment). After rewatering, leaf water potential recovered quickly (within 2 d) but g_l did not.

Additional key words: *Citrus limon*, elastic adjustment, leaf conductance, osmotic potential, water deficit, water potential.

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Abbreviations: C - control plants; E - bulk modulus of elasticity; g_l - leaf conductance; RWC_{tlp} - relative water content at turgor loss point; S - water-stressed plants; SLM - specific leaf mass; ψ_{md}, ψ_{pd}, ψ_{tlp} - midday, predawn and at turgor loss point, respectively, leaf water potential; ψ_{os} - leaf osmotic potential at full turgor.

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Introduction

The citrus trees are native of subtropical and tropical areas in eastern Asia. The Fino lemon tree is a Spanish cultivar widely cultivated in the south-eastern Mediterranean coast of Spain. The aridity of the climate conditions in this area requires irrigation for commercial crop culture, and the scarcity of water resources means the development of deficit irrigation strategies (Domingo *et al.* 1996).

Plants have developed many mechanisms to survive water deficit, including escape, tolerance, and avoidance of tissue water deficits. Avoidance of stress includes changes in leaf area, orientation and anatomy, among others (Jones 1980). Tolerance to stress involves at least two mechanisms, osmotic adjustment and changes in the elastic properties of tissues (Munns 1988, Savé *et al.* 1993). Most citrus crops are able to withstand considerable drought (Fereres *et al.* 1979). This resistance is demonstrated by the fact that evapotranspiration of well irrigated trees is lower than potential evapotranspiration, due, in part, to the low leaf conductances of citrus in arid climates (Hall *et al.* 1975). However, a better understanding of citrus adaptations to drought stress may help to optimise deficit irrigation management.

For these reasons, the aim of this paper was to identify the mechanisms of avoidance and tolerance to drought, which were operating in mature and young leaves of Fino lemon plants, when they were submitted to a cycle of severe water stress and recovery.

Materials and methods

Plants and culture conditions: The experiment was carried out on 2-year-old lemon trees (*Citrus limon* (L.) Burm. fil.) cv. Fino on sour orange (*Citrus aurantium* L.) rootstock, growing under field conditions in pots (40 cm diameter) filled with a mixture of clay loam top soil and peat, containing 4 % organic matter. Holed pots were buried in the soil in order to minimise increases in soil temperature. Plants were drip irrigated daily maintaining the soil matric potential at about -30 kPa (monitored with tensiometers placed at 20 cm depth) over a year. A routine fertilization programme was applied (100 g N, 60 g K_2O and 40 g P_2O_5 per plant and year). From the 17th June of the second year, control plants (C) were irrigated daily, as indicated, and water stressed plants (S) had water withheld for 13 d. In these latter plants the top of the pots were sealed with a plastic film and covered with soil in order to decrease the rate of development of water stress. Recovery of the stressed plants was carried out by reirrigation of pots to run off and their subsequent maintenance as for the control plants for a further 5 d. No root emergence from pots into the surrounding soil was observed. Design of the experiment was completely randomised with four replications. Four plants per replicate were used. A two-way *ANOVA* was performed and means were separated by $LSD_{0.05}$ range test. No rainfall was recorded during the experimental period. The climatic conditions were: maximum/minimum air temperatures of 30.5/18.5 °C, the relative humidity between 26 - 72 %, the average wind velocity at 2 m height 0.81 m s^{-1}, and the average class A pan evaporation 8.4 mm d^{-1}.

Measurements: Leaf water potential (Ψ_l) was measured on days 0, 4, 7, 13, 15 and 18 of the experimental period, at predawn (Ψ_{pd}) and at midday (12:00 solar time) (Ψ_{md}), on eight mature (> 4 months old) and eight young (≈1 months old) leaves from the south side of four plants per treatment, using a pressure chamber, following the recommendations of Turner (1988). Leaf conductance (g_l) was measured at midday on a similar number of leaves as Ψ_l, using a steady-state porometer. Dry and wet bulb temperatures were recorded on the dates of measurements, and vapour pressure deficit (VPD) was calculated. Photosynthetically active radiation (PAR) was measured at the ground surface within the plant-shaded area with a quantum sensor. Eight PAR readings were taken at 10 and 20 cm from the trunk at the four cardinal points on four plants per treatment. Also, PAR readings were taken outside the shaded area of the plants. Estimates of leaf osmotic potential at full turgor (Ψ_{os}), leaf water potential at turgor loss point (Ψ_{tlp}), bulk modulus of elasticity (E) and relative water content at turgor loss point (RWC_{tlp}) were obtained from pressure-volume analysis (Wilson *et al.* 1979, Ruiz-Sánchez *et al.* 1993) on days 0, 7, 13 and 15 of the experimental period. Specific leaf mass (SLM) was determined on the same dates and number of leaves as Ψ_l at midday, on samples of 12 leaf discs (18 mm in diameter) oven dried for 48 h at 80 °C.

Results

During the experimental period, leaf water potential at predawn (Ψ_{pd}) and at midday (Ψ_{md}) were constant in young and mature leaves of control plants (Fig. 1), Ψ_{md} being lower than Ψ_{pd}. In control plants, leaf water potential at predawn showed high and similar values (around -0.23 MPa) in both leaf types, whereas at midday Ψ_{md} for young leaves was significantly lower than for mature ones. From the beginning of the withholding of water, a progressive decline in Ψ_{pd} and Ψ_{md} values was observed, reaching in both cases minimum values of -5.5 MPa, with no significant differences between mature and young leaves. After rewatering the plants, Ψ_{pd} and Ψ_{md} values reached control levels rapidly (within 2 d).

Leaf conductance (g_l) in young and mature leaves for control plants showed a close dependence on vapour pressure deficit of the atmosphere (VPD), with high g_l associated with low VPD (data not shown: young leaves $g_l = 132.4 - 25.8$ VPD, $r = 0.96^{***}$; mature leaves $g_l = 82.5 - 16.2$ VPD, $r = 0.77^{*}$). From these equations, it is clear that young leaves had higher g_l than those of mature leaves for a given VPD.

From day 4 of the withholding period, an important reduction in g_l was observed in both leaf types, maintaining very low levels throughout the stress period (Fig. 2). The recovery of g_l after rewatering was slower than those of Ψ_{pd} and Ψ_{md}; 5 d were required to reach similar values to the control plants. Stressed plants showed leaf rolling and partial defoliation, which were evident at the end of the first week of the stress period. All these effects were indirectly evaluated by measuring the PAR transmission through the plant canopy. Control plants intercepted about 65 % of the incident PAR, whereas in stressed plants the PAR interception decreased as water stress progressed, reaching values of around 15 % at the end of the stress period (data not shown).

The osmotic potential at full turgor (Ψ_{os}) was lower in mature than in young leaves (Table 1). Ψ_{os} values in mature leaves from control plants were constant during the

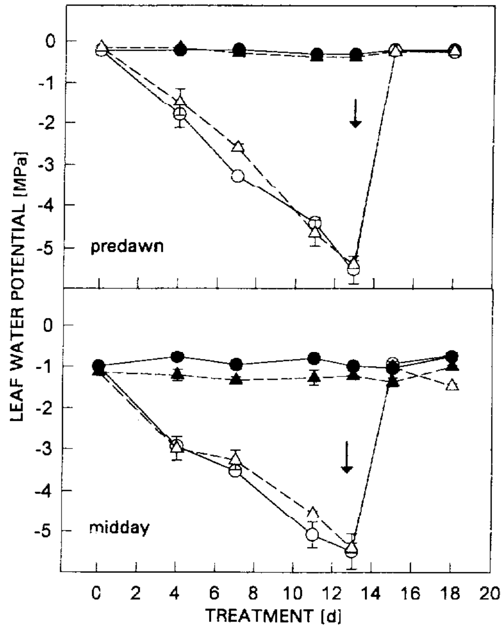

Fig. 1. Leaf water potential at predawn (Ψ_{pd}) and at midday (Ψ_{md}) of young (*triangles*) and mature (*circles*) leaves for Fino lemon plants in control (*closed symbols*) and stress (*open symbols*) treatments during the experimental period. *Arrows* indicate the beginning of the recovery period. *Vertical bars* represent S.E. of the mean (not shown when smaller than the symbols); $n = 8$.

Table 1. Leaf osmotic potential at full turgor (Ψ_{os}), leaf water potential at turgor loss point (Ψ_{tlp}), bulk modulus of elasticity (E) and relative water content at turgor loss point (RWC_{tlp}) of young and mature leaves for Fino lemon plants in control (C) and stress (S) treatments, before the beginning of the treatments (t-0), in the middle of the stress period (t-1), at the end of the stress period (t-2) and two days after rewatering (t-3). Data not shown correspond to leaves that there were not possible to obtain a complete PV curve due to water stress level.

Time	Treat.	Ψ_{os} [MPa]		Ψ_{tlp} [MPa]		E [MPa]		RWC_{tlp} [%]	
		young	mature	young	mature	young	mature	young	mature
t-0	C	-1.18 x	-2.11 y	-1.44 x	-2.60 y	16.44 x	15.33 x	86.86 x	87.08 x
t-1	C	-1.54 ax	-2.07 ay	-1.78 ax	-2.54 ay	17.16 ax	14.08 ax	85.40 ax	85.75 ax
	S	-1.54 ax	-2.19 ay	-1.87 ax	-2.66 ay	8.79 bx	13.97 ay	78.66 bx	84.79 ay
t-2	C	-1.63 x	-2.09 ay	-1.87 ax	-2.50 ay	15.72 x	15.72 ax	88.56 x	85.70 ax
	S		-1.98 a		-2.72 a		5.21 b		78.57 b
t-3	C	-1.60 ax	-2.08 ay	-1.87 ax	-2.52 ay	15.74 ax	15.78 ax	88.63 ax	85.40 ax
	S	-0.99 bx	-2.01 ay	-1.39 bx	-2.45 ay	15.48 ax	13.54 ax	90.39 ax	88.71 ax

Means with different letters for a given column (a,b) or a row (x,y) within sampling date are significantly different by $LSD_{0.05}$ test; $n = 4$.

experiment, whereas Ψ_{os} of young leaves showed a trend to decrease. No significant differences between treatments were found in either leaf type during the stress period. After rewatering, a significant increase in Ψ_{os} values was noted only in young stressed leaves. Leaf water potential at turgor loss point (Ψ_{tlp}) (Table 1) presented a similar trend to that observed for Ψ_{os}. Absent values correspond to stressed young leaves at the end of the stress period, which could not be adequately used for PV curve analysis due to the severity of the water stress.

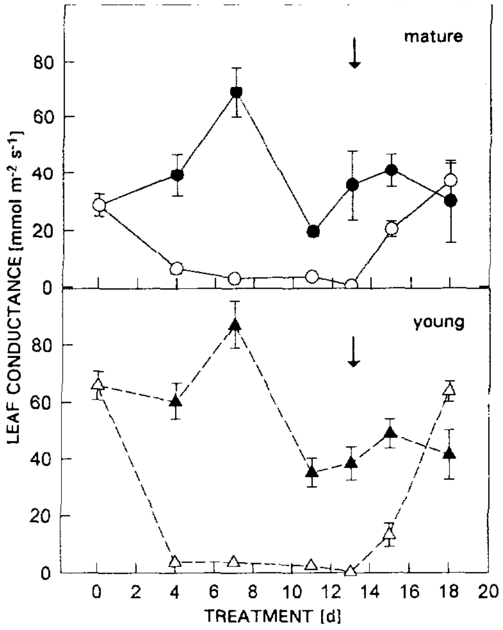

Fig. 2. Leaf conductance (g_l) at midday of young (*triangles*) and mature (*circles*) leaves for Fino lemon plants in control (*closed symbols*) and stress (*open symbols*) treatments during the experimental period. *Arrows* indicate the beginning of the recovery period. *Vertical bars* represent S.E. of the mean (not shown when smaller than the symbols); $n = 8$.

Values of bulk modulus of elasticity (E) were similar and constant in both leaf types of control plants during the experimental period (Table 1). Water stress induced a reduction in E, which was first apparent in young leaves. This water stress effect disappeared after rewatering. The relative water content at turgor loss point (RWC_{tlp}) behaved similarly to that of the bulk modulus of elasticity (Table 1).

Water stress did not affect specific leaf mass (SLM) of either leaf type (Fig. 3). Mature leaves had higher SLM (around 14 mg cm^{-2}) than young ones (around 10 mg cm^{-2}). The latter showed a significant decrease in SLM during the recovery period, reaching values of 7 mg cm^{-2} at the end of the experiment.

Moisture release curves were not affected by water stress (Fig. 4). Nevertheless, significant differences were found at Ψ_l below -1.6 MPa due to age difference between leaf types, with a given RWC being associated with a lower Ψ_l for the mature leaves.

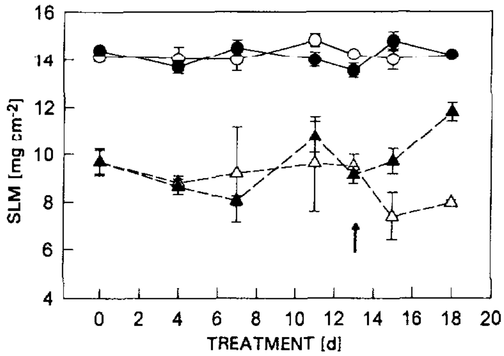

Fig. 3. Specific leaf mass (SLM) of young (*triangles*) and mature (*circles*) leaves for Fino lemon plants in control (*closed symbols*) and stress (*open symbols*) treatments, at midday, during the experimental period. *Arrow* indicates the beginning of the recovery period. Vertical bars represent S.E. of the mean (not shown when smaller than the symbols); $n = 4$.

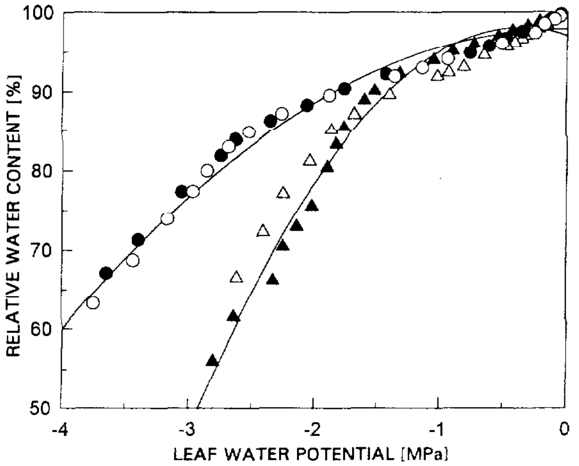

Fig. 4. Water release curves of young (*triangles*) and mature (*circles*) leaves for Fino lemon plants in control (*closed symbols*) and stress (*open symbols*) treatments. Data relate to a replicate of the middle of the stress period sampling date. Each point corresponds to a single pressure-volume observation.

Discussion

The high and constant Ψ_{pd} in control plants during the experimental period (Fig. 1) can be considered to reflect adequate irrigation practices, which is in accordance with the constant soil matric potential values (around -30 kPa). Predawn leaf water potential mainly depends on soil moisture conditions (Elfving *et al.* 1972). The rate of development of water stress was moderate (0.4 MPa per day for Ψ_{pd}) according to Hale and Orcutt (1987). The severity of water stress reached at the end of the withholding period induced similar Ψ_{pd} and Ψ_{md} values, suggesting that leaf water potential did not recover during the night. This was due to the depletion of volumetric soil water content, from 33.5 % (field capacity) to 14 % at the end of the stress period. The rapid recovery of leaf water potential after rewatering the plants (Fig. 1) has also been observed in orange plants subjected to severe water stress (Fereres *et al.* 1979).

The decrease in g_l values as a result of the increase in evaporative demand of the atmosphere (VPD) is a characteristic of citrus plants and is in agreement with the results obtained by Sinclair and Allen (1982). The higher leaf water deficits presented in young leaves of control plants, as indicated by lower Ψ_{md} values (Fig. 1), can be explained by the higher g_l in this leaf type (Fig. 2). Several authors have indicated that g_l decreases as a result of the ontogenetic changes that take place in the leaves, which include changes in the hormonal balance of the leaf itself, changes in membrane permeability and cell wall strength as leaves matured, as well as differences in stomatal physiology, leaf surface wax distribution and cuticular water loss rates of new and old leaves (Syvertsen *et al.* 1981, Solárová and Pospíšilová 1983). The fact that mature lemon leaves show higher SLM than young ones (Fig. 3) confirms these ideas.

The very low midday g_l values induced by water stress from the early phase of the stress period (from day 4 onwards) (Fig. 2) may be related to the low ψ_{md} (Fig. 1), which indicate that leaf turgor loss is in agreement with the Ψ_{tlp} values (Table 1). The delay in the recovery of g_l after rewatering, with respect to that of Ψ_l, indicated that stomatal closure was not a simple passive response to water deficit; thus, the pattern of stomatal behaviour under water stress is also related to hormonal changes in the leaf, such as abscisic acid increase and/or cytokinins decrease (Davies and Zhang 1991). Nevertheless, the partial stomatal closure after rewatering can be considered as a safety mechanism which allows the plant to regain full turgor more efficiently (Mansfield and Davies 1981).

The leaf rolling and partial defoliation which occurred at the end of the first week of the stress period, when the leaves presented Ψ_{pd} values around -3.0 MPa, can be considered a complementary mechanism to stomatal closure in regulating transpiration more efficiently.

Leaf Ψ_{os} in mature leaves from the control treatment (Table 1) indicated that Ψ_{os} did not change throughout the season, once the leaves were fully expanded and suberised. Similar results have been found in almond plants by Ruiz-Sánchez *et al.* (1993). The lower Ψ_{os} values in mature than in young leaves and the trend towards lower Ψ_{os} in young control leaves may be related to a natural ontogenetic process (Yoon and Richter 1990). The absence of significant differences between control and stressed leaves in Ψ_{os} values of both leaf types (Table 1) indicated that lemon plants were unable to develop osmotic adjustment in response to water stress.

The fact that Ψ_{os} increased in young leaves of stressed plants during the recovery period (Table 1) can be related to the effect of solute exclusion resulting from the water stress. The SLM decrease in the leaves during the recovery period (Fig. 4) is in line with this behaviour. Ψ_{os} increase, due to some depletion of vacuolar solutes in the process of leaf ageing, was found in senescent leaves of two evergreen shrubs (Karlic and Richter 1983) and almond trees (Ruiz-Sánchez *et al.* 1993).

Both leaf types showed a clear capacity to enhance the cell wall elasticity (E decreases) in response to water stress (Table 1). However, this mechanism of drought tolerance was insufficient to prevent tissue turgor loss because of the severity of the water stress reached (Fig. 1, Table 1). Elastic adjustment can play an important role in the maintenance of processes which are turgor-dependent, such as growth and photosynthesis, under water stress conditions (Bradford and Hsiao 1982, Munns 1988, Savé *et al.* 1993).

The RWC_{tlp} behaviour in both leaf types (Table 1) appeared to be controlled by E values. The RWC_{tlp} decrease during the stress period, which concurred with no change in Ψ_{os} (Table 1) could be explained by a significant increase in cell wall elasticity. An absence of significant differences between both leaf types in E values was recorded for lemon plants (Table 1); by contrast, it has been found an E decrease as a result of leaf ageing in almond trees (Ruiz-Sánchez *et al.* 1993) and in *Lycopersicon pennellii* (Sánchez-Blanco *et al.* 1991). The opposite behaviour has also been reported (Zimmerman 1978).

The lower slope in Ψ_l *vs* RWC relationship under -1.6 MPa in mature leaves (Fig. 4) might be induced by the higher apoplastic water content in mature leaves (Ruiz-Sánchez *et al.* 1993). This situation could indicate an adaptive feature since a given RWC was associated with a lower Ψ_l, thus allowing for a steeper gradient in Ψ between the leaf and the soil, favouring water absorption. This behaviour, together with the lower Ψ_{tlp} of mature leaves, can enable them to maintain turgor under moderate water stress conditions better than young leaves.

The results indicate that lemon plants respond to water stress essentially by developing drought avoidance mechanisms, such as stomatal closure, leaf rolling and partial defoliation. Lemon plants are also able to develop elastic adjustment as a drought tolerance mechanism. These plants did not show osmotic adjustment in response to moderate or severe water stress. On the other hand, mature lemon leaves are equipped with some adaptive mechanisms (higher midday leaf water potential and specific leaf mass, lower leaf conductance and leaf water potential at the turgor loss point), which might allow them to confront water deficits more efficiently than young leaves.

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