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Responses of *Quercus ilex* from different provenances to experimentally imposed water stress

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Abstract

Responses of *Quercus ilex* L. seedlings from three different localities in Italy to experimentally imposed drought stress were analysed. Predawn (Ψ_{pd}) and midday (Ψ_m) leaf water potential of stressed seedlings decreased on an average until -4.0 and -4.2 MPa, respectively, in the severe water stress. At the end of the severe water stress the relative water content (RWC) was 72.5 - 83.6 % and the photosynthetic rates (P_N) near zero. The critical threshold value of Ψ_{pd} for complete stomatal closure was from -4.0 to -4.5 MPa. The leaf damage after the severe water stress was significantly greater in seedlings originated from the acorns of climax area (45 % total leaf injured area and 40 % fallen leaves) than in the other seedlings (on an average 20.5 % total leaf injured area and 21 % fallen leaves).

Additional key words: drought stress, leaf conductance, leaf injury, net photosynthetic rate, relative water content, water potential, water use efficiency.

Introduction

Holm oak (*Quercus ilex*) is a deep-rooted evergreen sclerophyllous species widely distributed in the Mediterranean Basin (Barbero *et al.* 1992). It is able to maintain relatively high leaf water potential during drought showing early stomatal closure before serious alteration of the water status (Fotelli *et al.* 2000), and a high degree of co-ordination is apparent between stomatal behaviour and photosynthetic rates (Faria *et al.* 1998, Gratani 2000). During the year, the cumulative carbon assimilation of *Quercus ilex* is drastically reduced when CO_2 uptake is below 50 % of its optimal rates for long periods of time (Gratani *et al.* 2000); the critical temperature of the thermal inactivation of thylakoid functions in leaves of *Quercus ilex* has been estimated 45 - 55 °C (Méthy *et al.* 1997, Loreto *et al.* 1998). Drought stress is one of the most important limiting factor of carbon gain, as it induces stomatal closure reducing gas diffusion between the mesophyll and the surrounding atmosphere (García-Plazaola and Becerril 2000), particularly when water deficit is associated to high air temperatures and high irradiance (Peñuelas *et al.* 1998, Gratani and Bombelli 2000). Davis (1989) suggests that it is particularly important to study differences in adaptability to water stress at the seedling stage because of the highest mortality rate observed in seedling establishment of woody Mediterranean species. Physiological knowledge concerning individuals of *Quercus ilex* from different climates are important to forecast the species potential productivity to increasing drought stress.

The objective of this study was to analyse water relations and gas exchange of *Quercus ilex* seedlings from different localities in Italy in response to experimentally imposed water stress. Leaf injury after the stress period was also quantified. The hypothesis tested in this study was that *Quercus ilex* seedlings from the southern origin (drier areas) could have a higher intrinsic resistance to drought stress.

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Abbreviations: C - control plants; E - transpiration rate; E_A - Nago provenance; E_B - Castelporziano provenance; E_C - Frassanito provenance; g_{leaf} - leaf conductance to water vapour diffusion; P_N - net photosynthetic rate; RWC - relative water content; S - stressed plants; WUE - water use efficiency; Ψ_{pd} - predawn leaf water potential; Ψ_m - midday leaf water potential; $\Delta\Psi$ - daily difference of leaf water potential.

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Materials and methods

Experimental design: Experiments were conducted in a semi-controlled greenhouse of the Plant Biology Department of the University of Rome "La Sapienza" under natural irradiance up to 1500 μmol m^{-2}s^{-1} during the morning. Seedlings of *Quercus ilex* L. were grown in 32 cm diameter and 29 cm depth pots containing peat and soil (1:3); the soil comprised silt (5 - 8 %), clay (16 - 39 %) and sand (56 - 75 %) and pH was 7.2 -7.5. The seedlings under study were germinated from seeds of parent plants from three different localities in Italy: Nago (E_A), 45°55'N, 10°53'E, 260 m a.s.l., in the Garda Lake region at the northern limit of holm oak distribution area in Italy; Castelporziano (E_B), 41°45'N, 12°26'E near Rome, at sea level, at the centre of the distribution area; Frassanito (E_C), 40°13'N, 18°26'E near Lecce, at sea level, in a drier area of the south of Italy.

The desiccation experiments were conducted in the period May - July 2001 on seedlings from the three provenances. Water was withheld from 10 plants per provenance (E_AS, E_BS, and E_CS) to generate water stress, according to Filella *et al.* (1998). The well-watered control plants (10 seedlings per provenance, E_AC, E_BC, and E_CC) were watered regularly to field capacity. During the first experiment (it was referred to as mild drought stress) diurnal air temperature was 24 - 29 °C, and air humidity 70 % in the early morning and 40 % at midday; during the second experiment (it was referred to as severe drought stress) diurnal air temperature was 28 - 37 °C, and air humidity 50 % in the early morning and 25 % at midday.

Plant water relations: Predawn and midday leaf water potentials (Ψ_{pd}, Ψ_m) corresponding to the maximum and minimum diurnal values were measured at the beginning and at the end of each experiment on ten fully expanded leaves per provenance for control and stressed plants with a pressure chamber (*SKPM 1400, Sky Instruments*, Powys, UK). The daily difference of leaf water potential ($\Delta\Psi$) was calculated as the difference between Ψ_m and Ψ_{pd} (Damesin and Rambal 1995). Relative water content at predawn (RWC) was calculated at the same time as (FM-DM)/(TM-DM) × 100 (Lo Gullo and Salleo 1988) where FM was the fresh mass, DM the dry mass and TM the mass of fully hydrated samples. Leaf samples were hydrated until saturation for 48 h at 5 °C in the darkness and then dried in an oven at 90 °C until constant mass.

Leaf gas exchange: Net photosynthetic rate (P_N), transpiration H_2O rate (E), and leaf conductance to water vapour diffusion (g_{leaf}) were monitored during the experiments on ten fully expanded leaves of ten plants per provenance by an infrared CO_2 gas analyser (*ADC-LCA4*, Hoddesdon, UK) equipped with a leaf chamber *PLC4*. The instantaneous water use efficiency (WUE) was calculated as the ratio between photosynthetic and transpiration rates.

Leaf damage: At the end of the second experiment (severe water stress) the percentage of the injured leaf area was estimated by the presence of dark necrotic areas, according to Kyriakopoulos and Richter (1991). The surface of injured leaf area was determined by an *Image Analysis System* (*Delta-T Devices*, Cambridge, UK). Moreover, the number of the leaves fallen per plant was recorded.

Statistical analysis: All statistical tests were performed using a statistical software package (*Statistica, Statsoft*, Tulsa, USA). Differences in the considered traits were determined by analysis of variance (*ANOVA*). Regression analysis was used to explore relationships among the considered traits.

Results

Plant water relations: Leaf water potential of stressed plants decreased with increasing water stress: Ψ_{pd} and Ψ_m were -2.6 MPa and -3.4 MPa, respectively (mean of E_AS, E_BS and E_CS) at the end of the first experiment (Table 1). A major reduction of Ψ_m was observed at the end of the second experiment (-4.1 MPa in E_CS and -4.3 MPa in E_AS and E_BS).

Stressed seedlings showed a progressive reduction of $\Delta\Psi$ to the decrease of leaf water potential. In particular, at the end of the second experiment $\Delta\Psi$ was close to zero (0.1, 0.5 and 0.2 MPa in E_AS, E_BS and E_CS, respectively). $\Delta\Psi$ significantly correlated (r = 0.97, $P < 0.005$) with Ψ_{pd}.

RWC in stressed plants (Table. 1) tended to remain relatively stable under mild stress conditions: 91.3 and 87.8 % were the mean values of E_AS, E_BS and E_CS at the beginning and at the end of the experiment, respectively. A higher reduction of RWC during severe desiccation (second experiment) was observed: 85.9 - 88.1 % were the mean values of E_AS, E_BS and E_CS at the beginning, and 72.5 - 83.6 % at the end of the experiment.

Leaf gas exchange: In mild stress condition, P_N of stressed plants decreased of 98.5 % (mean of E_AS, E_BS and E_CS) at the fourth day respect to the first day (Fig. 1*A*); at the third day in severe stress condition P_N was zero in E_CS while in E_AS and E_BS plants released CO_2 (apparent respiration). Differences among E_BS and

E_CS were significant ($P < 0.01$). Leaf conductance decreased gradually, during the two experiments, from 150 to nearly 5 mmol (H_2O) m^{-2} s^{-1} (Fig. 1*B*); this g_{leaf} was typical for stomatal closure in sclerophylls (Larcher 2002). The relationship of g_{leaf} with Ψ_{pd} of control and stressed plants was an exponential (Fig. 2); the critical threshold values of Ψ_{pd} for complete stomatal closure were on an average from -4.0 to -4.5 MPa under the severe water stress.

The instantaneous water use efficiency (WUE; Fig. 3) showed a reduction from the beginning to the end of the two experiments and E_CS always showed higher values that the others. Stressed plants had significantly lower WUE values than the controls.

Leaf damage: The leaf damage after the imposed severe water stress was significantly greater in E_BS (45 % total leaf injured area, 40 % leaves fallen) than in E_AS (35 % total leaf injured area, 36 % fallen leaves) and especially E_CS (6 % total leaf injured area, 6 % fallen leaves).

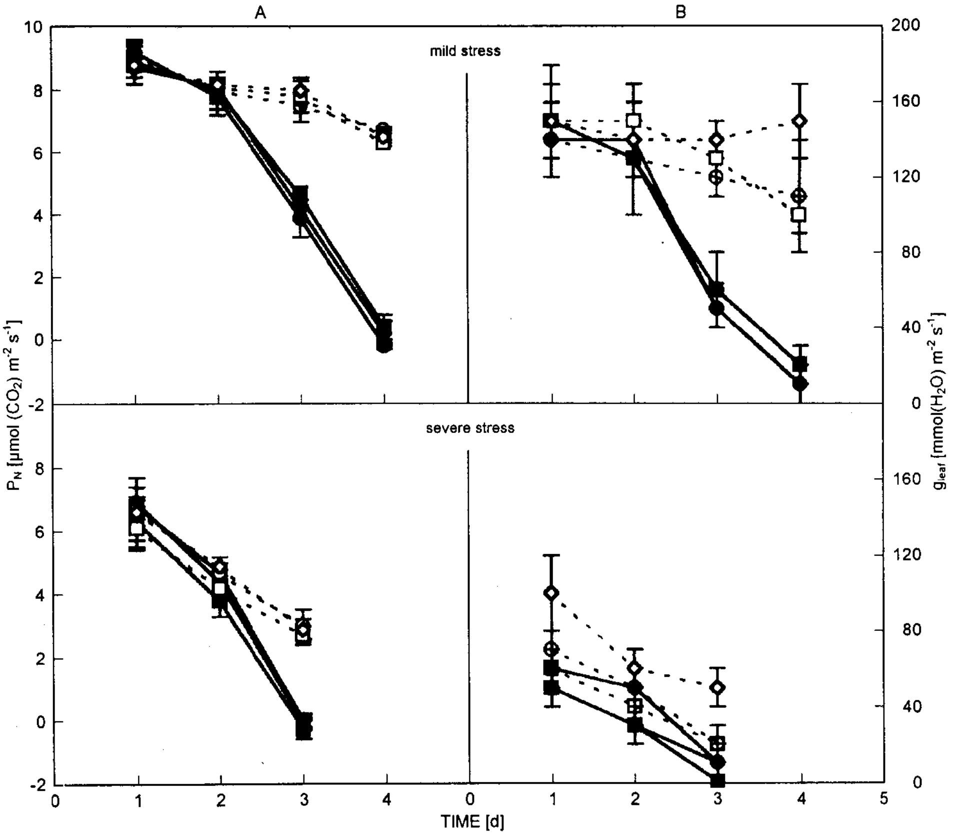

Fig. 1. Changes in net photosynthetic rates, P_N (*A*), and leaf conductance, g_{leaf} (*B*) of control (*open symbols*) and stressed (*close symbols*) plants from different localities (seedlings from Nago - *circles*, seedlings from Castelporziano - *squares*, and seedlings from Frassanito - *rhombs*) during the experiments. *Vertical bars* indicate standard deviation.

Discussion

In our experiments, even when water stress was at the maximum, $\Delta\Psi$ decreased with Ψ_{pd}, but Ψ_m remained higher than -4.3 MPa characterising *Quercus ilex* as a "regulator" species, according to Rambal (1992). *Quercus ilex* avoided irreversible cell damage by approaching Ψ_{pd} to Ψ_m in drought conditions. The closure of stomata protected the seedlings from exposure to severe water deficit; the critical threshold of Ψ_{pd} for stomatal closure of *Quercus ilex* seedlings in the severe water stress experiment averaged between -4 and -4.5 MPa, and the early decrease of stomatal conductance prevented irreversible cell dehydration contributing to the tolerance strategy, according to Larcher (1960) and Damesin and Rambal (1995). Moreover, drought-induced leaf fall was a mechanism for coping with extreme drought conditions (Kramer 1980).

Low photosynthetic rates did not ensure survival during prolonged stress periods (Peñuelas *et al.* 1998). The results of decreased P_N during short periods of water stress showed a different sensitivity of P_N of *Quercus ilex* seedlings of Nago, Castelporziano and Frassanito to imposed water stress. Interaction of high air temperatures

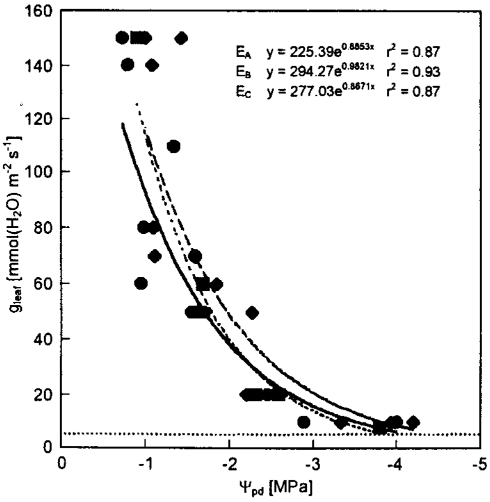

Fig. 2. Relationship between leaf conductance (g_{leaf}) and predawn leaf water potential (Ψ_{pd}) of control and stressed plants from different localities: E_A (seedlings from Nago - *circles*), E_B (seedlings from Castelporziano - *squares*), and E_C (seedlings from Frassanito - *rhombs*). *Dotted line* - threshold stomatal closure of sclerophylls.

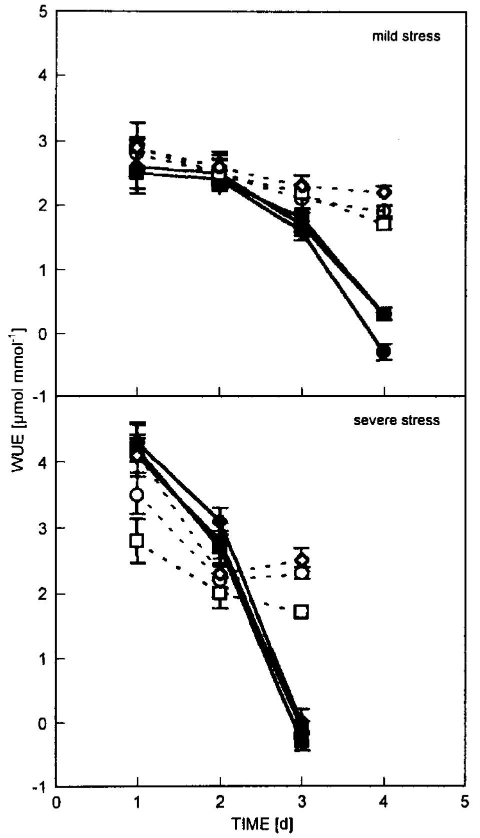

Fig. 3. Changes in water use efficiency (WUE) of control (*open symbols*) and stressed (*close symbols*) plants from different localities (seedlings from Nago - *circles*, seedlings from Castelporziano - *squares*, and seedlings from Frassanito - *rhombs*) during the experiments. *Vertical bars* indicate standard deviation.

Table 1. Predawn (Ψ_{pd}) and midday (Ψ_m) leaf water potential, daily difference of leaf water potential $\Delta\Psi$, and RWC measured at the beginning (b) and at the end (e) of each experiment of control (C) and stressed (S) plants of *Quercus ilex* from Nago (E_A), Castelporziano (E_B) and Frassanito (E_C). Means ± SD, *n* = 10.

Stress			Ψ_{pd} [MPa]	Ψ_m [MPa]	$\Delta\Psi$ [MPa]	RWC [%]
Mild	E_AC	b	-0.8 ± 0.05	-3.1 ± 0.27	2.3	92.0
		e	-1.3 ± 0.32	-3.2 ± 0.25	1.9	91.4
	E_BC	b	-0.9 ± 0.11	-2.2 ± 0.17	1.3	90.5
		e	-1.4 ± 0.40	-2.6 ± 0.21	1.2	89.8
	EcC	b	-0.7 ± 0.06	-2.6 ± 0.19	1.9	91.0
		e	-1.4 ± 0.30	-3.2 ± 0.21	1.8	90.5
	E_AS	b	-0.7 ± 0.07	-3.2 ± 0.29	2.5	92.3
		e	-2.9 ± 0.48	-3.3 ± 0.50	0.4	89.4
	E_BS	b	-0.7 ± 0.07	-2.7 ± 0.22	2.0	90.9
		e	-2.6 ± 0.37	-3.5 ± 0.35	0.9	85.2
	EcS	b	-0.6 ± 0.05	-2.9 ± 0.21	2.3	90.7
		e	-2.2 ± 0.35	-3.3 ± 0.40	1.1	88.8
Severe	E_AC	b	-1.6 ± 0.10	-3.3 ± 0.24	1.7	88.2
		e	-2.4 ± 0.09	-3.5 ± 0.17	1.1	86.6
	E_BC	b	-1.7 ± 0.12	-3.3 ± 0.28	1.6	85.7
		e	-2.3 ± 0.16	-3.4 ± 0.21	1.1	83.8
	EcC	b	-1.4 ± 0.09	-2.9 ± 0.22	1.5	87.6
		e	-2.3 ± 0.25	-3.3 ± 0.30	1.0	86.4
	E_AS	b	-1.6 ± 0.09	-3.8 ± 0.29	2.2	88.1
		e	-4.2 ± 0.28	-4.3 ± 0.31	0.1	81.9
	E_BS	b	-1.6 ± 0.08	-3.5 ± 0.38	1.9	85.9
		e	-3.8 ± 0.20	-4.3 ± 0.35	0.5	72.5
	EcS	b	-1.3 ± 0.09	-3.5 ± 0.23	2.2	87.1
		e	-3.9 ± 0.51	-4.1 ± 0.49	0.2	83.6

(up to 36 °C) with water deficit could be the reason of release of CO_2 at the end of the second experiment; Gratani *et al.* (2000) showed that the cumulative carbon assimilation available for plant growth was drastically reduced when leaf temperature was above 36 °C .

Quercus ilex seedlings from Castelporziano which had evolved under a climate with no winter stress and a superficial water-bearing stratum explored all year by roots showed a higher sensitivity to severe water stress than Frassanito seedlings evolved under naturally prolonged drought stress. These ones seemed to be the most tolerant seedlings to experimentally imposed water stress showing the highest RWC, P_N, WUE, and the lowest leaf injury. Nago seedlings from a climate with winter stress (air temperatures frequently below zero) and a mild summer stress seemed to be in the middle of a sensitivity scale of drought stress. Seedlings from Frassanito seemed to be more tolerant to desiccation experiments and they might provide a substantial advantage on drier sites and in response to the climatic change effects on Mediterranean ecosystems. Leaf water relations data provide a useful indication of the capacity of woody species to maintain growth, gas exchange and water use during drought (Nardini *et al.* 1996, Nilsen and Orcutt 1996, Rico *et al.* 1996, White *et al.* 2000).

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