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Influence of transient shade periods on the effects of drought on photosynthesis, carbohydrate accumulation and lipid peroxidation in sunflower leaves

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Abstract

The effects of a slow-imposing two-weeks soil drying period, and subsequent re-watering, on leaf water potential (Ψ), gas exchange rates, chlorophyll fluorescence and on the concentrations of malondialdehyde (MDA) and non-structural carbohydrates (starch, hexose and sucrose) were determined in mature leaves of sunflower plants growing under controlled environmental conditions. To assess how transient shade periods, associated with increased cloud cover, may influence drought-induced effects on carbon assimilation, measurements were carried out both in plants kept under the photosynthetic photon flux density (PPFD) prevailing during the growth period and stress imposition ($750 \mu\text{mol m}^{-2} \text{s}^{-1}$), and in plants subjected to a 5 hours-long period under a lower PPFD ($200 \mu\text{mol m}^{-2} \text{s}^{-1}$). In plants kept under high PPFD, Ψ , stomatal conductance (g), net CO_2 uptake rate (A), the quantum yield of photosystem II electron transport (Φ_e), the photochemical efficiency of open PSII reaction centres (F_v'/F_m') and

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the diurnal accumulation of total non-structural carbohydrates (TNC) were significantly depressed at the end of the soil drying period, whereas non-photochemical quenching (NPQ), the concentrations of MDA and the predawn pools of soluble sugars were found to increase. Under high-light level, drought-induced effects on lipid peroxidation, chlorophyll fluorescence parameters and gas exchange rates were fully reversed upon re-watering. However, the inhibition of diurnal accumulation of TNC still persisted two days following re-watering, suggesting that carbohydrate export rates were enhanced following stress relief. An overall positive effect was found upon transferring water-stressed plants to low light level, as indicated by the increases in Ψ , intrinsic water use efficiency (A/g), Φ_e and F_v'/F_m' , as well as the reversal of drought-induced enhancement of both NPQ and MDA concentration. Despite g being similar in shaded well-watered and re-watered plants, the latter exhibited net CO_2 uptake rates below those found in well-watered leaves under the same light conditions, together with a diurnal decrease in the concentration of TNC that was mainly attributable to the depletion of starch and sucrose. These results indicate that contrasting with the positive effects of shading on water-stressed plants, low PPFD may negatively affect the recovery of net photosynthesis following stress relief.

Keywords: drought; *Helianthus annuus*; non-structural carbohydrates; photosynthesis; lipid peroxidation; shade

Abbreviations: A , net carbon dioxide uptake rate; C_i , intercellular carbon dioxide concentration; ETR, electron transport rate; F_v/F_m , maximal photochemical efficiency of photosystem II; F_v'/F_m' , photochemical efficiency of open PSII reaction centres; g , stomatal conductance for water vapour diffusion; MDA, malondialdehyde; NPQ, non-photochemical quenching; PPFD, photosynthetic photon flux density; PSII, photosystem II; q_p , photochemical quenching; TNC, total non-structural carbohydrates; Φ_e , quantum yield of photosystem II electron transport; Ψ , leaf water potential

1. Introduction

Water deficit is a major constraint to plant growth and productivity, one of the earliest effects of soil drying being the decline in photosynthetic carbon assimilation, resulting from stomatal and mesophyll limitations (Lawlor, 2002; Flexas et al., 2004). However, the extent of drought-induced depression in photosynthesis, and its underlying causes, may be dependent on the superimposition of other environmental stress conditions (Chaves, 1991). As water stress episodes in field conditions are often accompanied by strong light, the interaction between high irradiance and drought has been investigated in a number of studies. Ögren and Öquist (1985) found no interaction between drought and irradiance, provided the leaves were properly light-acclimated. However, other studies have shown that water deficits predispose the photosynthetic apparatus to photoinhibition, and drought-induced inhibition of photosynthetic activity is exacerbated when leaves are dehydrated under strong light (Björkman and Powles, 1984; Masojídek et al., 1991; Behera et al., 2002). In contrast, Valladares and Pearcy (2002) recently reported that the capacity of plants to withstand a severe drought might be decreased under shade conditions, rather than being enhanced.

On studies undertaken to assess the influence of light regime on the effects of drought, light level is often kept constant throughout the experimental period. In contrast, the photosynthetic photon flux density varies temporally under field conditions. Cloud cover is one of the major causes of varying sunlight intensity under field conditions, and the incidence of transient shade periods, associated with increased cloud cover, increases during precipitation events. Therefore, re-watering of rain-fed plants is expected to occur under irradiances lower than those prevailing during growth and water deficit development. The impact of those transient decreases in irradiance on carbon assimilation under drought stress and after stress relief has been scarcely addressed. In a study in which sunflower plants were subjected to a days-long shade period during water deficits imposition, Ben et al. (1987) found that the extent of drought-induced reduction in the CO₂-saturated rate of photosynthesis was greater in leaves kept at high light than in shaded leaves, and no drought-induced changes in photochemistry was detected in the latter. Results obtained in a recent work (Sofa et al., 2004), in which the shading treatment was extended throughout the entire re-watering period, also showed that partial shade alleviates the oxidative load on leaves, and leads

to a faster recovery of photosynthesis. Another likely effect of decreased irradiance would be the limitation of drought-induced accumulation of sugars (Munns and Weir, 1981). Carbohydrate build-up in leaves can lead to decreased expression of photosynthetic genes, and hence to a depression in photosynthetic activity (Paul and Foyer, 2001). Therefore, shade-induced limitation in sugar accumulation is expected to alleviate end-product inhibition of photosynthesis which has been reported to occur under drought conditions (Souza et al., 2004). The above mentioned studies point out to a positive effect of shading on photosynthetic activity under drought conditions, but it is conceivable that the depression in the capacity of the leaves to produce carbohydrates may limit the recovery of growth rate after drought stress relief.

When leaves are subjected to low light, a decreased respiratory rate is essential to allow a positive carbon balance. Because acclimation of leaf respiratory rates to the new light environment may take a few days (Noguchi et al., 2001), temporary carbon imbalance is likely to occur following sudden decreases in irradiance, as respiration continues at high rates typical for high-light grown leaves. This may contribute to explain why switching high light-grown leaves to low irradiance conditions has been found to be associated with a substantial decrease in foliar levels of total non-structural carbohydrates (Veneklaas and den Ouden, 2005), and to negatively affect leaf instantaneous water use efficiency (Fay and Knapp, 1995). However, the relative magnitude of the negative effects of short-term (minutes-long) shade periods on transpiration and photosynthesis, and hence the way water use efficiency is affected, has been found to be dependent on the watering regime (Fay and Knapp, 1998). These results highlight the importance to proceed studies on the influence of sudden decreases in light level on plant responses to drought stress.

The present work was undertaken in order to contribute to a better understanding of the short-term effects of low irradiance on the response of plants to drought. With that purpose, the effects of soil drying, and subsequent rewatering, on water status, gas exchange rates, chlorophyll fluorescence, lipid peroxidation and accumulation of non-structural carbohydrates were determined in mature leaves of sunflower plants, either kept under growth irradiance or subjected to a 5 hours long period of partial shade. To our knowledge, the simultaneous effects of temporary shading on leaf gas exchange rates, chloroplast photochemistry, leaf oxidative damage, and sugar accumulation in water-stressed leaves has not been assessed so far.

2. Materials and methods

2.1. Plant material and growth conditions

Helianthus annuus L. seeds (cv. Giant) were soaked overnight and allowed to germinate on wet tissue paper, in darkness. They were then placed in 3 l pots filled with a mixture of unfertilised peat and vermiculite (1:1, v/v). The plants (one per pot) were grown in a controlled-environment cabinet (Fitoclima 16.000 EHVP, Aralab, Portugal) under a photoperiod of 12 h, with light provided by incandescent and fluorescent lamps, supplying a photosynthetic photon flux density (PPFD) of about $750 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the top of the plants. The temperature was 18/25°C (night/day) and the relative humidity was 70/60% (night/day). Watering was done using a complete nutrient solution.

From sowing until the beginning of water deficit imposition, all the pots were regularly brought to field capacity (each two days). The onset of water deficit imposition took place 19 days after sowing, and afterwards the pots were watered on a daily basis. Water stress was imposed by replacing only about half the water lost by evapotranspiration (determined gravimetrically). After 17 days of soil drying, the water-stressed pots were re-watered to field capacity. In the case of the plants referred to as well-watered, all the water lost by evapotranspiration was replaced by watering throughout the entire experimental period. Measurements and sampling of plants subjected to water deficit took place 10 and 17 days after the onset of soil drying. Re-watered plants were sampled 24 and 48 h following rewatering. Well-watered plants were sampled the days preceding sampling of water-stressed plants.

Two light treatments were imposed in each watering regime: half of the plants were kept in the growth chamber where growth took place (high-light treatment); the plants subjected to the low-light treatment were transferred, at the end of the dark period that preceded the day of sampling, to another growth chamber in which PPFD was lower ($200 \mu\text{mol m}^{-2} \text{s}^{-1}$). The temperature regime was the same on the two growth chambers.

A different set of plants was sampled on each day (five plants per treatment), and all measurements were undertaken on fully-expanded, non-senescent leaves. Unless otherwise stated, all measurements were carried out 5 h after the lights were turned-on (midday).

2.2. Measurements of leaf water status, gas exchange rates and chlorophyll fluorescence

Leaf water status was assessed by measuring leaf water potential (Ψ) using a pressure chamber (PMS Instruments, Corvallis, OR, USA).

Stomatal conductance for water vapour diffusion (g), net carbon dioxide uptake rate (A), intercellular CO_2 concentration (C_i) were determined using a portable gas exchange measuring system (HCM-1000, H. Walz, Effeltrich, Germany).

Chlorophyll fluorescence measurements were undertaken immediately before the lights were turned-on (predawn), and at midday, using a portable pulse-modulated fluorometer (PAM-2000, H. Walz, Effeltrich, Germany). The maximal photochemical efficiency of photosystem II (PSII) was estimated, at predawn, by the fluorescence ratio F_v/F_m of dark-adapted leaves, calculated from F_0 (basal fluorescence) and F_m (maximal fluorescence), with F_v being the variable fluorescence. The photochemical efficiency of open PSII reaction centres (F_v'/F_m') was calculated by measuring the same parameters as above on light-adapted leaves (F_0' and F_m'). After recording the steady-state value of fluorescence of light adapted leaves (F_s), a saturating flash was imposed to determine the maximal level in the light-adapted state (F_m'). Afterwards, a black cloth was placed over the leaf, and a far-red pulse (3 s) was provided in order to fully oxidise PSII during the measurement of the minimal level in the light-adapted state (F_0'). The effective PSII quantum efficiency in light-adapted leaves (ϕ_e) was calculated by the $(F_m' - F_s)/F_m' = (\Delta F / F_m')$ ratio (Genty et al., 1989). As described by Krall and Edwards (1992), the electron transport rate (ETR) was estimated as $\text{ETR} = (\Delta F / F_m') \times \text{PPFD} \times 0.5 \times 0.84$. The leaf absorbance used was 0.84 because that is the most common value for C_3 plants (Björkman and Demmig, 1987). The photochemical quenching (q_p), which was used as an estimate of the fraction of open centres, was calculated according to Bilger and Schreiber (1986), whereas the Stern-Volmer equation (Cornic, 1994) was used to calculate non-photochemical quenching (NPQ), which is an estimate of the thermal energy dissipation at PSII.

2.3. Quantification of malondialdehyde and non structural carbohydrates

The level of oxidative damage to lipids was assessed by determining the concentration of malondialdehyde (MDA), a product of the oxidation of

polyunsaturated fatty acids. Extraction of leaf material, measurements and procedures to determine the amount of MDA equivalents, corrected for interfering compounds in leaf extracts, were performed as described by Hodges et al. (1999). In short, leaf material (approximately 100 mg fresh mass) was powdered in liquid nitrogen and suspended in 1 ml 20% trichloroacetic acid (TCA). Each sample had two replicates, two blanks and a reference. Replicates were obtained after homogenization and centrifugation (3000 g at 4°C, during 10 min), by the addition of 500 µl of supernatant to 1.5 ml of a thiobarbituric acid (TBA) solution made in TCA (0.5% TBA in 20% TCA, v/v). In the case of the blanks the sample was replaced by 500 µl of 20% TCA. The reference was obtained by adding 500 µl of supernatant to 1.5 ml 20% TCA. All the samples, references and blanks were submitted to a heat/cool cycle and again centrifuged (10 min at 10000 g and room temperature), the absorbances at 532 nm, 600 nm and 440 nm being determined in the supernatant using a spectrophotometer Shimadzu UV-160. MDA content was calculated by correcting for compounds other than MDA which absorb at 532 nm, such as anthocyanins, by subtracting the absorbance at 532 nm of the reference. Absorbances measured at 600nm and 440nm allowed to take into account interference due to non-specific turbidity and carbohydrates, respectively, as described by Hodges et al. (1999).

Soluble sugars and starch were quantified in leaf discs punched at predawn and at midday. Soluble sugars were extracted from intact leaf discs, with 80% (v/v) ethanol, at 80°C, for 20 min. Sucrose, fructose and glucose were then measured in the ethanol extract using a spectrophotometric enzyme-coupled assay, as described by David et al. (1998). After the extraction of soluble sugars, the leaf discs were then used for starch analysis. After being subjected to a washing step, the leaf discs were homogenised with water, the resulting suspension was autoclaved, and subsequently subjected to hydrolysis (Stitt et al. 1989). Glucose released from starch hydrolysis was quantified using a colorimetric assay modified to be used in 96 well plates, as described in Hatterscheid and Willenbrink (1991). Starch in each sample, expressed as glucose equivalents, was determined using glucose solutions as standard. The sum of soluble sugars and starch is referred to as total non-structural carbohydrates (TNC).

2.4. Data analysis

Statistical analysis and graphic display were performed with SPSS (Release 12.0, SPSS Inc., Chicago, IL) and SigmaPlot (Version 8.02, SPSS Inc., Chicago, IL) software packages, respectively.

Data from leaf water status, leaf gas exchange rates, chlorophyll fluorescence and lipid peroxidation were subjected to one-way ANOVA after testing for normality and homogeneity of variance. If the ANOVA yielded a significant F value ($P < 0.05$) then the four individual means of the water-stress and re-watering treatments were compared to the well-watered control using a Dunnett post-hoc test. In case of heterogeneity of variances the Kruskal-Wallis test was used for nonparametric analysis of variance, and differences between watering regimes means were tested with Kruskal-Wallis multiple-comparison Z-value tests. At each watering regime, independent-samples *t*-tests were computed to compare the calculated means between plants kept under high-light and those transferred to low-light conditions.

Data from non-structural carbohydrates were also subjected to one-way ANOVA. At each watering regime, differences between the two light regimes and predawn means were tested using Dunnett post-hoc tests. In addition, differences at predawn between water-stress and re-watering treatments and the well-watered control were tested in the same way. In case of heteroscedastic data, Kruskal-Wallis nonparametric ANOVA and Kruskal-Wallis multiple-comparison tests were used instead.

All differences were considered to be statistically significant if $P < 0.05$.

3. Results

3.1. Leaf water status

Restricting the water supplied through watering resulted in the development of a substantial leaf water deficit in the case of plants kept under high-light: when compared to well-watered plants, leaf Ψ decreased only by 0.2 MPa during the first 10 d of limited water supply, but at the end of soil drying period Ψ of water-stressed leaves was 0.9 MPa lower than in well-watered ones. Following rewatering Ψ quickly recovered to control levels.

As shown in Fig. 1, decreasing irradiance positively affected leaf water potential, shade-induced increase in Ψ being higher in the case of droughted plants than in well-watered ones. Consequently, the difference in Ψ between well-watered and droughted plants, although not eliminated, was attenuated upon switching to low irradiance conditions: the difference in Ψ between well-watered and water-stressed plants did not exceed 0.6 MPa in the case of plants transferred to low light conditions.

3.2. Leaf gas exchange rates

In plants kept under high-light conditions, neither stomatal conductance (g) nor net carbon dioxide uptake rate (A) were significantly affected after 10 days of soil drying. However, as shown in Fig. 2, by the end of the soil drying period, A and g were strongly depressed, the values of both parameters decreasing to about 10% of values determined in well-watered plants. No significant drought-induced change was detected at the level of the inter-cellular concentration of carbon dioxide (C_i) measured under high-light conditions.

In the case of the more severely stressed plants, stomatal conductance and net photosynthesis increased by about 50% and 75%, respectively, in response to shading (Fig. 2), although those changes were not statistically significant. In contrast, upon transferring well-watered and moderately stressed plants to low-light conditions, g decreased by about 35%, the concomitant depression in A being even more accentuated: net photosynthesis of shaded leaves of well-watered plants was below 30% of values determined under high-light conditions. As a consequence of the opposite response of A to shading exhibited by well-watered and severely-stressed plants, the negative effects of soil drying on A were alleviated under low-light conditions: when compared to well-watered plants under the same light conditions, severely stressed plants kept under high-light exhibited a 10 fold decline in A , whereas under shade conditions A was only halved in response to soil drying. The light conditions also influenced the way A/g ratio and C_i were affected by soil drying: in severely-stressed plants under shade conditions, A/g ratio increased by 10-fold (data not shown) and C_i was halved (Fig. 2) compared to well-watered plants, whereas those two variables did not vary significantly in response to water deficits in the case of the plants kept under the light level prevailing during the growth period and stress imposition.

Despite the strong drought-induced depression in photosynthesis and stomatal conductance, the negative effects of soil drying on A and g , measured under growth irradiance, were no longer detected 24 hours following re-watering. In contrast, re-watered plants transferred to low-light conditions exhibited net CO_2 uptake rates significantly lower than those determined in well-watered plants under the same light conditions, despite full stomatal reopening following re-watering (Fig. 2). Actually, on the day following rewatering, net CO_2 uptake rates determined in shaded leaves were 25% lower than those determined in severely stressed plants under the same light conditions (Fig. 2). The different extent of photosynthetic recovery following stress relief was associated with the enhancement of the negative effects of shading on net photosynthesis following re-watering: upon transferring plants to low-light conditions, A decreased to about 25% and 10% of the values determined in plants kept under high-light in the case of well-watered and re-watered plants, respectively.

3.3. Chlorophyll fluorescence

Despite the severity of drought-induced reduction in A , the maximal photochemical efficiency of PSII, estimated by the fluorescence ratio F_v/F_m of dark-adapted leaves, was close to maximum (varying between 0.84 and 0.87), irrespective of the watering and light regimes to which the plants were subjected (Fig. 3).

The photochemical activity of light adapted leaves also remained unaffected after 10 days of restricted in water supply. However, in more severely-stressed plants kept under growth irradiance, the photochemical efficiency of open PSII reaction centres (F_v'/F_m' ratio), the quantum yield of PSII electron transport (ϕ_e), and the electron transport rate (ETR) decreased significantly, although moderately: the F_v'/F_m' ratio was decreased by 10%, whereas the extent of drought-induced depression in ϕ_e and ETR reached about 15%. Drought-induced restriction in ϕ_e was also accompanied by a moderate increase in the reduction state of Q_A , as indicated by a 5% decrease in photochemical quenching (q_p), whereas non-photochemical quenching (NPQ) increased by about 40%, indicating the enhancement of thermal energy dissipation at the PSII. Drought-induced changes in chlorophyll fluorescence were fully reversible upon water deficit relief, all the fluorescence parameters returning to pre-stress levels on the day following re-watering.

As shown in Fig. 3, upon transferring the plants to low-light conditions, ϕ_e , the F_v'/F_m' ratio and q_p increased, whereas NPQ decreased, the extent of shade-induced changes in fluorescence parameters increasing with the severity of water stress. Consequently, when measured under low irradiance conditions, neither quenching coefficients of fluorescence nor the photochemical efficiency of PSII were significantly affected by the watering regime.

3.4. Lipid peroxidation

In the case of plants kept under high-light, by the end of the soil drying period the foliar concentration of malondialdehyde increased 37% above values found in leaves of well-watered plants, but drought-induced increase in lipid peroxidation was fully reversible following re-watering (Fig. 4).

Transferring the plants to low-light conditions did not significantly affect the foliar concentration of MDA, except in the case of the more severely stressed leaves, in which MDA concentration decreased by about 20% in response to decreased PPFD. Therefore, under low-light conditions, the concentrations of MDA in leaves of well-watered and water-stressed leaves were not significantly different.

3.4. Non structural carbohydrates

Soil drying was accompanied by a two-fold increase in the concentration of total non-structural carbohydrates determined at the end of the night period. As shown in Fig. 5, the above mentioned drought-induced accumulation of carbohydrates was mainly attributable to the build-up of soluble sugars: by the end of stress imposition, leaves of water-stressed plants exhibited a two-fold and ten-fold increase in the concentration of sucrose and hexose, respectively, when compared to well-watered plants. The concomitant 50% increase in the concentration of starch was not statistically significant, but the concentration of this insoluble carbohydrate increased following re-watering, reaching values about two-fold higher than those determined in well-watered plants by the end of the night that followed stress relief. Two days following re-watering the predawn pools of both hexose and starch returned to pre-stress levels, but significant differences between well-watered and re-watered plants still persisted as to their predawn concentrations of sucrose.

In the case of plants kept under growth irradiance, the concentration of TNC in well-watered leaves increased by two-fold from predawn to midday (Fig. 5). The above mentioned carbohydrate build-up was mainly attributed to the accumulation of starch and hexose, which concentrations increased by two- and six-fold, respectively, from predawn to midday. As shown in Fig. 5, by the end of the soil drying period no significant difference was found between predawn and midday concentrations of TNC, due to drought-induced abolishment of diurnal build-up of starch and decrease in the extent of hexose accumulation. The diurnal accumulation of TNC still persisted two days following re-watering (Fig. 5).

In the case of well-watered and water-stressed plants transferred to low irradiance conditions, no significant difference was detected between predawn and midday values of starch and soluble sugars concentrations. However, on the day following re-watering, the concentrations of TNC were approximately halved, from predawn to midday. The referred decrease in the concentration of carbohydrates was associated with the diurnal depletion of starch and sucrose, the latter still persisting two days following re-watering (Fig. 5).

4. Discussion

4.1. Effects of water deficits in plants kept under growth irradiance conditions

Although in the restriction in water supply was extended for more than two weeks, leaf Ψ never dropped below -1.5 MPa (Fig. 1), indicating a level of leaf water deficit less severe than that reported for field-grown sunflower plants (Freedman et al., 1991; Panković et al., 1999). Despite that, by the end of the soil drying period, the plants kept under high-light exhibited a significant increase in foliar concentration of MDA (Fig. 4). Since MDA is a product of lipid peroxidation (Hodges et al., 1999; Sofó et al., 2004), these results indicate that water deficits imposed to sunflower plants resulted in a moderate oxidative stress at the whole leaf level, similarly to what has been reported for other species (Behera et al., 2002; Jiang and Zhang, 2002).

The production of reactive oxygen species, and hence the occurrence of oxidative stress, may be promoted when severe limitation of net CO₂ uptake lead to an imbalance between the photochemical activity of PSII and the electron requirement for photosynthesis (Foyer et al., 1994). As shown in Fig. 2, net CO₂ uptake rate was

strongly depressed in response to soil drying. In previous studies in which sunflower plants have been subjected to similar levels of water deficits, drought-induced inhibition of net photosynthesis has been attributed either to stomatal limitation (Quick et al., 1992) or to non-stomatal factors (Ben et al., 1987; Maury et al., 1996; Panković et al., 1999). In the present work, even though stomatal conductance was severely limited, C_i tended to increase, rather than decreasing (Fig. 2), suggesting that increased resistance to CO_2 diffusion is not sufficient to explain the observed decline in A . This contention is supported by the chlorophyll fluorescence data (Fig. 3) which evidenced that the PSII photochemistry was affected by water deficits, as indicated by the decrease in the quantum yield of PSII electron transport (Φ_e).

However, the strong drought-induced depression in A (Fig. 2) did not result in non-reversible photoinhibitory damage to PSII reaction centres, since the ratio F_v/F_m did not change in response to soil drying (Fig. 3). Activation of nonradiative dissipative processes, such as thermal dissipation of excess excitation energy associated with the xanthophylls cycle, is one of the protective mechanisms that plants use to avoid photoinhibitory damage (Demmig-Addams and Adams, 1992). As shown in Fig. 3, drought-induced depression in Φ_e was largely a result of decreased efficiency of excitation energy capture by open PSII reaction centres (F'_v/F'_m), whereas the concomitant changes in q_p were insignificant. A decrease in F'_v/F'_m may reflect light-induced non-photochemical quenching (Baker, 1991). In accordance, a significant rise in NPQ was observed in the more severely stressed plants kept under growth irradiance (Fig. 3), providing evidence that part of the excitation energy in PSII antennae was dissipated as heat.

Since incident PPFD was kept constant, drought-induced decrease in ϕ_e was accompanied by a similar decrease in the rate of electron transport (Fig. 3). However, the extent of ETR depression was much smaller than the concomitant decline in net CO_2 uptake rates (Fig. 2). Similar results were obtained in other studies (Flexas et al., 1998; Wingler et al., 1999; Osório et al., 2004), and were interpreted as indicating that the partitioning of electron flow by pathways other than carbon assimilation were enhanced under drought stress. Apart from carbon assimilation, photorespiration, oxygen reduction through the Mehler-peroxidase reaction, and nitrate reduction are the main alternative sinks for electron flow through PSII. Nitrate reduction is not a likely candidate for consuming excess electrons under drought stress conditions, because a

strong depression in nitrate reductase activity occurs when sunflower are subjected to water deficits (Azedo-Silva et al., 2004). The results obtained in the present work do not allow the direct assessment of the relative contribution of photorespiration and oxygen reduction to sustain electron flow through PSII. However, the maintenance of high C_i values in droughted plants kept under high-light (Fig. 2) do not support the occurrence of drought-induced promotion of photorespiration. Under these circumstances, oxygen uptake by the Mehler-peroxidase pathway is likely to become the predominant electron sink (Biehler and Fock, 1996; Kitao et al., 2003).

In accordance with the strong drought-induced depression in A (Fig. 3), the extent of carbohydrates accumulation during the day was much higher in the leaves of well-watered plants than in leaves of severely stressed plants, but the latter exhibited higher predawn levels of TNC than well-watered plants (Fig. 5). The increase in the predawn pool of carbohydrates observed in severely stressed plants is likely to result from decreased rates of export, which have been reported to be inhibited under water deficits conditions (Quick et al., 1992; Pelleschi et al., 1997). Drought-induced inhibition of export rates apparently persisted during the first night following re-watering, since the pool of foliar TNC determined by the end of that night did not decrease below values determined at midday, before the plants were re-watered (Fig. 5). Afterwards however, carbohydrate export rates were apparently promoted by re-watering. This contention is supported by the observation that, in contrast to well-watered plants, re-watered plants exhibited no daytime carbohydrate build-up (Fig. 5), although net CO_2 uptake rates were similar on the two groups of plants (Fig. 2). That apparent increase in daytime export rates may be related with increased sink demand for photo-assimilate due to growth recovery after stress relief.

Water deficits imposed in the present work also affected the contribution of soluble sugars and starch to the total pool of carbohydrates: as shown in Fig. 5, in response to soil drying, the contribution of starch to the predawn pool of TNC decreased, and the diurnal starch build-up was abolished. These results are in agreement with previous studies showing that water deficits inhibit starch synthesis and induce a shift in the partitioning of newly formed carbohydrates towards soluble sugars (Freeden et al., 1991; Zrenner and Stitt, 1991; Quick et al., 1992). In accordance with Zrenner and Stitt (1991), who found that the inhibitory effect of drought on starch synthesis was rapidly reversed following rehydration, by the end of the first night after re-watering starch concentration increased above values found in severely stress plants, this increase

in starch content being proportional to the concomitant decrease in the concentration of soluble sugars (Fig. 5). These results may be interpreted as indicating that a redirection of carbon towards starch occurs soon after re-watering.

4.2. Influence of shading on the effects of soil drying and subsequent re-watering

The results obtained in the present work show an overall positive effect of short-term shading on droughted plants. In accordance with Sofó et al. (2004), who evidenced the ability of shading in limiting drought-induced oxidative damage in field-grown olive trees, in the present work drought-induced increase in lipid peroxidation was fully reversed upon the transfer of water-stressed sunflower plants to low-light conditions (Fig. 4). In addition, no drought induced-inactivation of the photochemical activity of PSII was detected in sunflower plants transferred to low-light conditions (Fig. 3), similarly to what has been previously found in others studies with this plant species (Ben et al., 1987; Panković et al., 1999).

Shade-induced reversal of drought-induced inactivation of photochemical efficiency was accompanied by increased intrinsic water use efficiency, as evidenced by C_i being approximately halved following the transfer of severely stressed plants to low irradiance conditions (Fig. 2). These results are apparently contradictory with previous reports indicating that shading negatively affects water use efficiency (Fay and Knapp, 1995). However, these apparent contradictory results may be conciliated taking into account that, similarly to what been previously found by Fay and Knapp (1998), the results obtained in the present work show that the effects of shading on C_i and A/g are dependent on the watering regime: as shown in Fig. 2, in contrast with what occurred with severely stressed plants, when well-watered, moderately stressed and re-watered plants were transferred to shade conditions, net photosynthesis was inhibited to a higher extent than stomatal conductance, resulting in increase C_i and decreased A/g .

Contrasting with the positive effects of shading on water-stressed plants, low irradiance may negatively affect the recovery of net CO_2 uptake rates following re-watering. As shown in Fig. 2, stomatal reopening following re-watering occurred irrespective of the light conditions to which the leaves were subjected, but the recovery of A after stress relief was strongly dependent on light conditions: whereas full recovery of net photosynthetic rate occurred on the first day after re-watering on plants kept under growth irradiance, net CO_2 uptake rates determined in re-watered plants

transferred to low-light did not surpassed half the values determined in well-watered plants under a similar light level.

Since the persistence of drought-induced inhibition of A following rehydration were restricted to measurements performed in plants transferred to low-light, we might hypothesise that respiratory processes were enhanced following re-watering. According to Cornic and Fresneau (2002), when leaves are dehydrated under limiting light an absolute increase in photorespiration is expected. However, drought-induced increases in photorespiration have been attributed to depleted C_i due to stomatal closure (Wingler et al., 1999; Cornic and Fresneau, 2002; Noctor et al., 2002), and in the present work stomata fully reopened and C_i returned to pre-stress levels following re-watering, irrespective of the light regime (Fig. 2). Therefore, even if photorespiration had been increased during the period of low g , it should be expected to return to control values following re-watering and concomitant stomatal reopening. As to mitochondrial respiration in the light, Haupt-Herting et al. (2001) reported that this process is inhibited by water stress in tomato plants, but to our knowledge, dark respiration in re-watered plants has not been assessed so far. However, increased demand for maintenance respiration may be expected during re-watering, in order to support the repair of dehydration-induced damages, including oxidative damage to lipid, as apparently happened in the present experiments, taking into account the observed increase in foliar levels of MDA (Fig. 4). In addition, the growth component of respiration is also expected to increase, as soon as growth restarts following the amelioration of plant water status.

It is note worthy that, under low-light conditions, the total pool of foliar carbohydrates was halved from predawn to midday on the day following re-watering (Fig. 5). This depletion of foliar carbohydrate can not be entirely explained by the negative effect of shading on carbon assimilation, since re-watered plants under low-light exhibited A values low but positive (Fig. 2). Therefore, these results suggest an enhancement of carbohydrate export rates following re-watering, as already suggested by the data obtained on plants kept under high-light. It is important to note that these results were obtained by measurements performed in a different set of plants each day, whereas field-grown rain-fed plants may be subjected to several consecutive cloudy days during rehydration periods. It should deserve future investigation the possibility of the cumulative effects of prolonged shading during re-watering leading to foliar carbohydrate starvation, which in turn may limit growth recovery.

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Fig. 1

Leaf water potential (Ψ) determined in sunflower plants well-watered (WW), after 10 days (S10) or 17 days (S17) of soil drying, and following 24 h (R1) or 48 h (R2) of re-watering. Means for plants transferred to low-light conditions (dashed bars) that are significantly different ($P < 0.05$) from those for plants kept under high-light (white bars) are marked with *. Values shown are mean \pm standard error of five replicates, except for the well-watered controls in which $n = 10$.

Fig. 2

Stomatal conductance for water vapour diffusion (g), net carbon dioxide uptake rate (A) and intercellular carbon dioxide concentration (C_i) determined in sunflower plants well-watered (WW), after 10 days (S10) or 17 days (S17) of soil drying, and following 24 h (R1) or 48 h (R2) of re-watering. Means for plants transferred to low-light conditions (dashed bars) that are significantly different ($P < 0.05$) from those for plants kept under high-light (white bars) are marked with *. Values shown are mean \pm standard error of five replicates, except for the well-watered controls in which $n = 10$.

Fig. 3

Maximal photochemical efficiency of PSII (F_v/F_m), photochemical efficiency of open PSII reaction centres (F_v'/F_m'), quantum yield of photosystem II electron transport (Φ_e), electron transport rate (ETR), photochemical quenching (q_p), and non-photochemical quenching (NPQ) determined in sunflower plants well-watered (WW), after 10 days (S10) or 17 days (S17) of soil drying, and following 24 h (R1) or 48 h (R2) of re-watering. Means for plants transferred to low-light conditions (dashed bars) that are significantly different ($P < 0.05$) from those for plants kept under high-light (white bars) are marked with *. Values shown are mean \pm standard error of five replicates, except for the well-watered controls in which $n = 10$.

Fig. 4

Concentrations of malondialdehyde (MDA) determined in leaves of sunflower plants well-watered (WW), after 10 days (S10) or 17 days (S17) of soil drying, and following 24 h (R1) or 48 h (R2) of re-watering. Means for plants transferred to low-light

conditions (dashed bars) that are significantly different ($P < 0.05$) from those for plants kept under high-light (white bars) are marked with *. Values shown are mean \pm standard error of five replicates, except for the well-watered controls in which $n = 10$.

Fig. 5

Concentrations of starch, hexose, sucrose and total non-structural carbohydrates (TNC) determined in leaves of sunflower plants well-watered (WW), after 10 days (S10) or 17 days (S17) of soil drying, and following 24 h (R1) or 48 h (R2) of re-watering. Means for concentrations determined at midday, in plants kept under high-light (white bars), and on those transferred to low-light conditions (dashed bars), that are significantly different ($P < 0.05$) from means determined at predawn (black bars) are marked with *. Values shown are mean \pm standard error of five replicates, except for the well-watered controls in which $n = 10$.









