

Shade mitigates photoinhibition and enhances water use efficiency in kiwifruit under drought

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Abstract

We tested the hypotheses that a reduction of incident light of 50 % over sun-acclimated leaves of water stressed kiwifruit (*Actinidia deliciosa* var. *deliciosa*) would (1) reduce stomatal limitations to carbon supply and (2) mitigate the inactivation of the primary photochemistry associated with photosystem (PS) II, thereby this increases carbon gain and water-use efficiency (WUE). Groups of field-grown vines were either shaded or left naturally exposed and subjected to progressive water stress in order to study moderately and severely droughted vines, while other groups were well irrigated. Daily variations in leaf gas exchange and midday chlorophyll (Chl) *a* fluorescence were determined once plants had -0.6 MPa (moderate stress) and -1.0 MPa pre-down leaf water potential (severe stress). Variations in Chl pigment content and specific leaf area (SLA) are also discussed. Results reveal that 50 % shade application maintained efficiency of PSII close to 0.8 even under severe drought so that to prevent its large decline (0.65) recorded in sunlit leaves. Under moderate stress level stomata behaviour dominated upon metabolic impairments of PSII. Reduction of irradiance increased WUE (15–20 %) in droughted vines, representing a valuable intervention to save photosynthetic apparatus and improve WUE in vines experiencing typical Mediterranean summer stresses.

Additional keywords: carbon gain; excessive radiation; photosynthesis; semi-arid condition; shade; stomatal limitation; water use efficiency.

Introduction

In natural ecosystems plant species segregate mainly across light gradients as predicted by the light partitioning hypothesis (Valladares *et al.* 2005). However, distribution at a global scale of some cultivated crops may be affected also by economic pressures. This is the case of kiwifruit (*Actinidia deliciosa* var. *deliciosa*, C.F. Liang et A. R. Ferguson) which originates from habitats characterised by high humidity, abundant rainfall (1 200–1 800 mm year⁻¹), long frost-free growing season (230–260 days) and by only a moderate intensity of sunlight (Ferguson 1984). In spite of these preferences, because of its commercial significance, kiwifruit is a widespread irrigated crop species also in semi-arid Mediterranean areas where summers are dry and irradiation typically high. In Southern Italy, currently the first world kiwifruit producer (FAO Statistic Division 2008), during a summer day irradiance may easily reach 1 800–

2 000 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ PPFD (photosynthetic photon flux density) for several hours. Photosynthesis in kiwifruit leaves of high-PPFD grown vines is light-saturated at 960 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ PPFD (Greer and Halligan 2001) being approx. at one-half of the usual midday summer irradiance.

The photosynthetic processes represent the carbohydrate source for plants, such a mechanism is mainly driven by irradiance and largely regulated by stomatal behaviour. However, at levels above the photosynthetic saturation point excessive radiation may reduce photosystem (PS) II efficiency (*i.e.* photoinhibition) (Demmig-Adams and Adams 1992) resulting in a stomata-independent restriction of carbon acquisition.

Stomata responses to environmental stimuli (*e.g.* evaporative demand, light and carbon dioxide, soil water content) have been investigated for a long time (Mansfield

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Abbreviations: Chl – chlorophyll; *E* – leaf transpiration; F_0 – minimal fluorescence of dark-adapted state; F_m – maximum fluorescence of dark-adapted leaves; g_s – stomatal conductance; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; PSII – photosystem II; SLA – specific leaf area; VPD – leaf-to-air vapour pressure deficit; Ψ_w – leaf water potential.

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et al. 1981, Lösch *et al.* 1981, Davies *et al.* 1981, Tardieu and Simonneau 1998). Nevertheless, under reduced soil water conditions, whether photosynthesis is limited through stomatal closure or by metabolic impairment is still debated (Flexas and Medrano 2002). In well irrigated kiwifruit vines, excess radiation can rise leaf temperature as much as 5–9 °C above air temperature resulting in poor carbon acquisition due to lower stomatal conductance (g_s), (Buwalda *et al.* 1992). Conversely, under co-occurring severe drought (*i.e.* –1.0 MPa pre-dawn leaf water potential, Ψ_w) it has been observed that limitations in carbon supply are due predominantly to metabolic impairments of PSII (*i.e.* photoinhibition) (Montanaro *et al.* 2007). The extent of the drought level (*i.e.* moderate or severe) seems to determine the origin (stomatal or metabolic) of carbon supply limitations (Flexas and Medrano 2002). However for moderately stressed kiwifruit (*i.e.* around –0.6 MPa pre-dawn Ψ_w), whether stomatal limitations dominate upon any metabolic injury of photosynthesis have not been elucidated.

Under a changing climate (increased temperatures and decreased precipitation) in order to limit yield penalties, the production should be sustained by interventions able to increase both carbon gain and water-use efficiency (Parry *et al.* 2005).

In some environments, reduced irradiance could be beneficial for leaf activity and plant survival (Knapp and Smith 1990). Physiological and structural leaf responses to various irradiance-drought scenarios have shown that shade could ameliorate (or at least not aggravate) the

drought impact in oak (Quero *et al.* 2006). However, it has been observed that a long-term shade period could dramatically lower photosynthetic capacity and yield in olive plants (Gregoriou *et al.* 2007).

At leaf scale, stomatal behaviour is also the primary control of plant water-use determining a large fraction of transpired water (Green *et al.* 2006). A number of studies have demonstrated that reducing excessive irradiance may increase WUE in apricot (Nicolás *et al.* 2005), grape and citrus species (Jifon and Syvertsen 2003, Alarcón *et al.* 2006). The effect of shade application in kiwifruit has been accessed mainly for fruit quality purpose (Snelgar and Hopkirk 1998, Montanaro *et al.* 2006).

Consequences of limited soil water availability on kiwifruit physiology have been investigated for a long time (Judd *et al.* 1989, Gucci *et al.* 1996), while there is still limited information existing on physiological and morphological leaf traits as modified by shade-drought interaction. Identification of the mechanisms (stomatal or non-stomatal) which have the largest contribution to carbon assimilation and water-use under different irradiance-water scenarios would be helpful and offer opportunities to create new applied conservative interventions. Therefore, we tested the hypothesis that reducing PPFD over sun-acclimated leaves by about 50 % would (1) reduce stomatal limitations to carbon supply and (2) mitigate the inactivation of the primary photochemistry associated with PSII. Thereby, this increases both carbon gain and WUE in vines experiencing typical Mediterranean summer stresses.

Materials and methods

Plant material and experimental design: Trials were carried out in Southern Italy at the “Pantanello” Agricultural Experimental Station near Metaponto (N 40° 23' E 16° 46') on 12-year-old own-rooted field-grown kiwifruit vines (*Actinidia deliciosa* A. Chev. C.F. Liang *et al.* R. Ferguson var. *deliciosa*) (cv. Hayward) during the summer of 2004. Vines were Pergola trained (494 plants ha⁻¹) with N-S row orientation.

Two groups were formed (each composed of 10 mature vines): one group was irrigated (IR), while the other was subjected to drought (D). Half of the vines in each group were left naturally exposed to full sunlight (sun) and half shaded (shade). The resulting treatments were: vines under irrigation and receiving full sunlight (IR_{sun}) or artificially shaded (IR_{shade}), and vines under drought receiving full sunlight (D_{sun}) or shaded (D_{shade}). Each treatment was replicated three times in the field.

During the experiment, control (irrigated) vines were regularly microjet-irrigated (wetting the whole soil surface area), approximately every 4–5 days on an evapotranspirative demand basis, in order to maintain soil moisture levels uniformly at around 90 % of field capacity. From July 17, progressive soil-water depletion was applied by withholding irrigation. In order to study

both moderately and severely stressed vines, measurements were performed 17 and 25 days after withholding irrigation (*i.e.* on August 4 and 12, respectively).

The shade treatment over vines was achieved by a neutral, transpiring cloth (591WO Ombraverde 50, Arrigoni, CO – Italy) causing a 50 % reduction in incident light. The shade cloth was placed 5 days after irrigation and withheld on 3 m high wooden frames approximately 1 m above the canopies. Radiation spectral distribution above and under the net was measured at the beginning of the experiment at 12:00 h on a clear day using a portable spectroradiometer (LI-1800, Li-Cor Inc., Lincoln, NE, USA).

Light measurements were made using quantum sensors (Model SKP 215, Skye Instruments Ltd., Llandrindod Wells, UK) placed close to the upper canopy layer (3 × treatment) to measure the incident PPFD. Air temperature and humidity were also monitored using HUMITER 50Y sensors (Vaisala, Helsinki, Finland) (3 × treatment). These were disposed close to the light sensors. All sensors were connected to a datalogger (CR10, Campbell Scientific, Utah, USA), which was programmed to record at 60 s intervals and to compute and store averages at 15 min intervals. The leaf-to-air

vapour pressure deficit (VPD) was calculated from air temperature and relative humidity values according to Goudriaan and van Laar (1994). Soil volumetric water content was measured at a depth of 30 cm using Time Domain Reflectometry equipment (*Trase System mod. 6050X1, Soil Moisture Equip. Corp.*, Santa Barbara, CA, USA) approx. every 2–3 days. Measurements were always performed at midday (11:00–12:00 h). Moisture levels (v/v) in irrigated and droughted soils are the averages of measurements at three points. Plant water status was assessed through leaf water potential (Ψ_w) measured pre-dawn and at different times of the day correspondingly to gas exchange measurements. Leaf water potentials were measured on three leaves per vine ($3 \times$ treatment) similar to those used for gas exchange analyses (see below) using a *Scholander* pressure chamber (*PMS Instrument*, Corvallis, OR, USA) pressurized with nitrogen according to the procedure recommended by Turner (1981).

Gas exchange and Chl *a* fluorescence: Leaf gas exchange and Chl *a* fluorescence measurements were performed once plants were moderately and severely droughted (i.e. pre-dawn Ψ_w close to -0.6 and -1.0 Mpa, respectively). Net photosynthetic rate (P_N), transpiration rate (E) and stomatal conductance (g_s) were measured using a portable gas exchange system *ADC-LCA4* operating at a flow rate of $200 \mu\text{mol s}^{-1}$ under the prevailing environmental condition. The *ADC* system was equipped with a *PLC4B* chamber (*ADC Inc.*, Hoddesdon, Hertfordshire, UK). Measurements were performed on 8 fully expanded leaves per treatment distributed on 4 terminating fruiting shoots from three plants, at least two records per leaf were taken. Leaf temperature was measured adaxially during gas exchange using the thermocouple installed in the *PLC4B* chamber. Diurnal gas exchange measurements were carried out always on the same leaves starting early in the morning (07:00 h) till the late afternoon, approximately every 2–3 hours. Between measurements the gas analyser and the chamber were placed in shade under aluminium foil to minimize exposure to high temperature.

Modulated Chl *a* fluorescence was measured at

midday (from 13:00 to 14:00 h) on the same leaf used for gas exchange measurements using a pulse amplitude modulation fluorometer *PAM-2000* (*Heinz Walz GmbH*, Effeltrich, Germany) connected to a personal computer with data acquisition software *DA-2000* (*Walz*). Measurements of the minimal (F_0) and maximal (F_m) level of fluorescence were made from leaves (adaxial side) maintained in the dark (30 min, dark leaf clip *Walz*). Maximal fluorescence was achieved by applying a brief saturating light pulse ($5\,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR). The maximal photochemical efficiency of PSII (F_v/F_m), where $F_v = F_m - F_0$, was then calculated. For all Chl *a* fluorescence and gas exchange measurements, leaves from the upper layer of the canopy were used, which were horizontally positioned as a consequence of the training system.

Chl pigment and SLA analyses: Chl *a* and *b* were determined on 3 leaves per plant ($3 \times$ treatment) sampled at the end of each measurement-day in order to analyse moderately and severely stressed leaves. Analyses were performed on a bulk of three discs (25 mm diameter) per leaf. After centrifugation of *N,N*-dimethylformamide (DMF) extracts, the absorbance of the supernatant at 625, 647, and 664 nm was determined by an *UV-VIS Beckman DU-50* spectrophotometer (*Beckman Instruments, Inc.*, Fullerton, CA, USA). The spectrophotometer had the spectral slit-width routinely set at 4 nm, and was calibrated in this experiment using 100 % DMF Chl-free as blank. The amount of Chl was then calculated by the Moran's (1982) formulae (Eqs. 6,7) and expressed per unit of leaf area. On the same leaves, two discs were sampled and dried to constant mass (48 h at 60°C) using a ventilated drying cabinet (*FED 400, WTB-Binder*, Tuttlingen, Germany) to determine SLA.

Statistical analyses: The effects of irradiance availability (exposed-shade treatments) as well as soil water content (irrigated-droughted treatments) on physiological and morphological leaf parameters were tested by means of *ANOVA* using the *STATISTICA*® 6.0 statistical package (*StatSoft Inc.*, Tulsa, OK, USA). Data are presented as means \pm SE (n - number of measurements).

Results

Environmental conditions and leaf characteristics: Irradiance, air temperature and air humidity were typical of Mediterranean summer conditions. Mean maximum PPFD at midday (11:00–12:00 h) on measurement days was about $1\,740 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ and was reduced by about 50 % as a result of shading (Fig. 1). The analysis of radiation spectral distribution showed that the application of the shading net did not determine qualitative variations of radiation (see inset of Fig. 1). In parallel, VPD reached its maximum between 12:00 and 15:00 h being noticeably higher in droughted canopies (26 % and

32 % on August 4 and August 12, respectively) (Fig. 1). Shading slightly alters VPD in both irrigated and droughted vines, on an average basis it was 3–4 % higher in exposed treatment during the hottest hours of the day.

The pre-dawn Ψ_w at 17 and 25 days after water withholding reached the mean values of -0.6 MPa (stress level I) and -1.0 MPa (stress level II), respectively (Fig. 2). The D_{sun} leaves were, on average, 1 to 3°C warmer than the D_{shade} leaves depending on stress level, while in the case of irrigated vines leaf temperatures were similar between irradiance levels (Table 1).

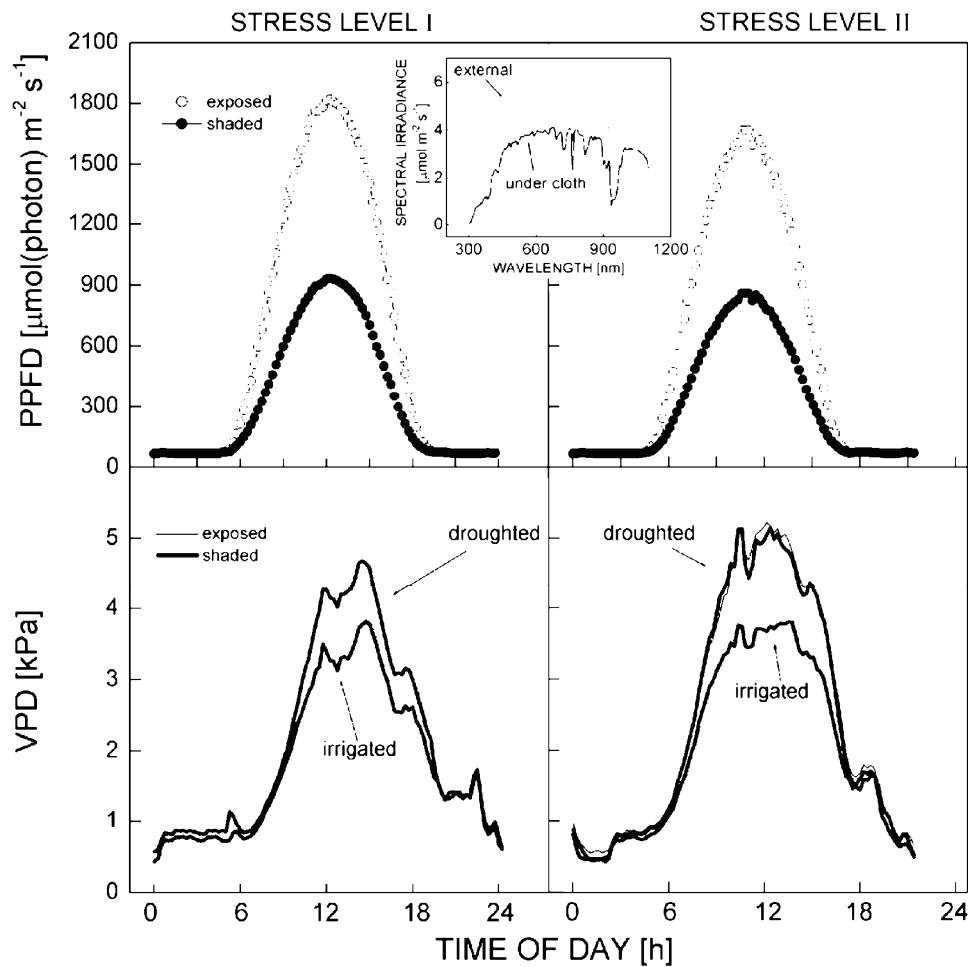


Fig. 1. Diurnal course of the incident light (PPFD, $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$) and VPD (kPa) in exposed (\circ , *light line*) and shaded (\bullet , *bold line*) canopies of irrigated and droughted vines during the stress level I (*left column*) and the stress level II (*right column*). The inset shows the spectral irradiance ($\mu\text{mol} \text{m}^{-2} \text{s}^{-1}$) above and under the cloth. Each line represents the average of 3 sensors.

Table 1. Specific leaf area (SLA), air and adaxial leaf temperature (T) measured early in the morning (07:00 h) and at midday (13:00 h) in irrigated (IR), moderately (stress level I) and severely (stress level II) water-stressed leaves (D) of vines exposed (sun) and artificially shaded (shade). Within a column, lowercase letters indicate statistically different means ($p < 0.05$). $n = 18$ (SLA); $n = 3$ (air T); $n = 16$ (leaf T).

	stress level I			stress level II		
	T [°C]	SLA [$\text{dm}^2 \text{g}^{-1} \text{DM}$]		T [°C]	SLA [$\text{dm}^2 \text{g}^{-1} \text{DM}$]	
	07:00 h	13:00 h		07:00 h	13:00 h	
air	25.1 ^a	31.7 ^a		27.2 ^a	34.6 ^a	
IR _{sun}	26.6 ^a	33.0 ^b	1.32 ^a	28.7 ^a	36.5 ^a	1.33 ^a
IR _{shade}	26.7 ^a	33.4 ^b	1.34 ^a	29.8 ^{ab}	36.2 ^a	1.36 ^a
D _{sun}	28.5 ^b	35.7 ^c	1.51 ^b	30.3 ^b	39.7 ^c	1.54 ^b
D _{shade}	29.1 ^b	34.9 ^c	1.58 ^b	30.7 ^b	36.5 ^a	1.63 ^b

Detailed analysis of Chl pigments in fully expanded leaves reveals that Chl *a* and *b* were slightly affected by shade (about 10 % increase) in moderately droughted leaves. In irrigated leaves under shade Chl *a* and *b* were 18 % and 29 % higher than exposed respectively.

Maximum amount of pigments was detected in shaded leaves of severely stressed vines being 32 % (Chl *a*) and 44 % (Chl *b*) higher than Chls of sunlit leaves respectively (Fig. 3).

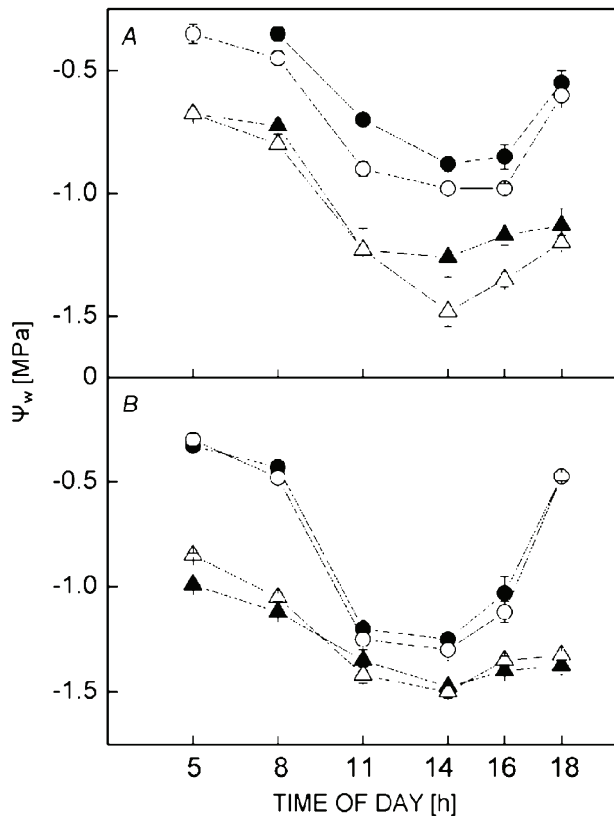


Fig. 2. Diurnal course of the leaf water potential (Ψ_w) during (A) August 4 (stress level I) and (B) August 12 (stress level II) in irrigated (circle) and droughted (triangle) vines. In both figures solid symbol (●, ▲) – shaded, open symbol (○, △) – exposed. Data are means \pm SE ($n = 9$).

Photosynthetic apparatus activity: As expected, transpiration (E) was strongly affected by PPFD and VPD exhibiting minimal values early in the morning and reaching the highest values at midday corresponding to maximum PPFD and VPD (Fig. 4A,B). Additionally, as a general trend, E values of shaded leaves were above those of exposed in all leaf types. Leaves of all treatments exhibited a marked midday depression in net assimilation rate (P_N) even though PPFD reached its maximum value (Fig. 4C,D). In general, shade application increased carbon uptake rates in both moderately and severely droughted vines. That is, on a daily average basis P_N of shaded leaves was 40 % and 47 % higher than sunlit leaves for stress level I and II, respectively, whereas in irrigated vines P_N values were affected to a lesser extent (± 15 %) by shade imposition (Fig. 4C,D). Stomatal conductance in droughted leaves was relatively high (and more responsive) to shade during stress level I being approximately 30 % (daily average) higher than that of exposed leaves (Fig. 4E). Whilst, at stress level II stomatal conductance was quite stable throughout the day

($\sim 0.12 \text{ mol(H}_2\text{O)} \text{ m}^{-2} \text{ s}^{-1}$), however shade application had remarkably prevented the huge drop of g_s (43 %) detected at midday in D_{sun} leaves (Fig. 4F).

The WUE of droughted vines diminished during the day reaching minimal values of about $1\text{--}2 \text{ } \mu\text{mol(CO}_2\text{)} \text{ mmol(H}_2\text{O)}^{-1}$ at 13:00 h, and thereafter a slight increase was observed in the afternoon (Fig. 6). Among water stressed leaves, the highest WUE was detected early in the morning (07:00 h) in leaves under shade being about 7.5 and $4 \text{ } \mu\text{mol(CO}_2\text{)} \text{ mmol(H}_2\text{O)}^{-1}$ for stress levels I and II, respectively. Cloth application caused WUE to be higher than that of unshaded leaves during the majority (4/5) of measurements we performed (Fig. 5).

In both droughted and irrigated vines, low light availability induced the midday F_v/F_m to be higher in shaded leaves (*i.e.* more efficient photochemistry) than sunlit leaves. However, differences were statistically significant ($p < 0.05$) only on August 12. Leaves under severe water deficit as well as high irradiance showed a remarkable low F_v/F_m value being equal to 0.65 (Fig. 6).

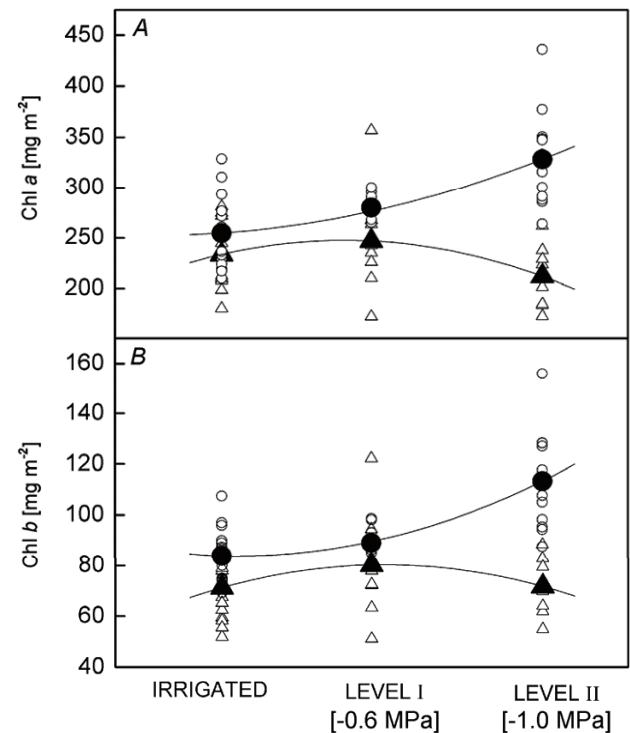


Fig. 3. Values of (A) chlorophyll (Chl) a (mg m^{-2}) and (B) Chl b (mg m^{-2}) measured in irrigated and stressed leaf tissues from sunlit (triangle) and shaded (circle) vines. Closed symbols (●, ▲) represent the average values of the single analyses (○, △). Irrigated values were from leaves taken at the same point in time when droughted vines reached the stress level I and II. The lines (a power curve fitted to the individual measurements) should be taken as indicative only.

Discussion

Plant water status and leaf traits: Generally, only few leaves (<5 % on a per plant basis) of severely droughted plants exhibited relevant symptoms of injury (*e.g.* necrotic spots). However, those utilised for all tests were absolutely intact. In all treatments Ψ_w decreased during the day reaching a minimum at 14:00 h showing a typical anisohydric behaviour (Tardieu and Simonneau 1998) (Fig. 2). For irrigated vines, Ψ_w dropped at midday more markedly on the second day of measurement (August 12) compared to the first (*i.e.* -1.25 and -1.0 MPa respectively). Such a difference could be explained considering that on an integrated daily basis VPD was

10 % higher on August 12 (Fig. 1). As a consequence, kiwifruit must set a more pronounced water potential gradient along the soil-root-leaf-atmosphere *continuum* to uptake water and securing the water it needs to meet transpiration demands (Green *et al.* 2006). Shade application induced Ψ_w to be 13 % and 8 % less negative (on a daily average basis) for irrigated and water-stressed vines respectively, but only during the first day of measurements (Fig. 2). Reduced water potentials may suggest a more efficient water supply toward the shaded canopy (Syvertsen 1985). However, this efficiency vanished 8 days later once vines were severely stressed.

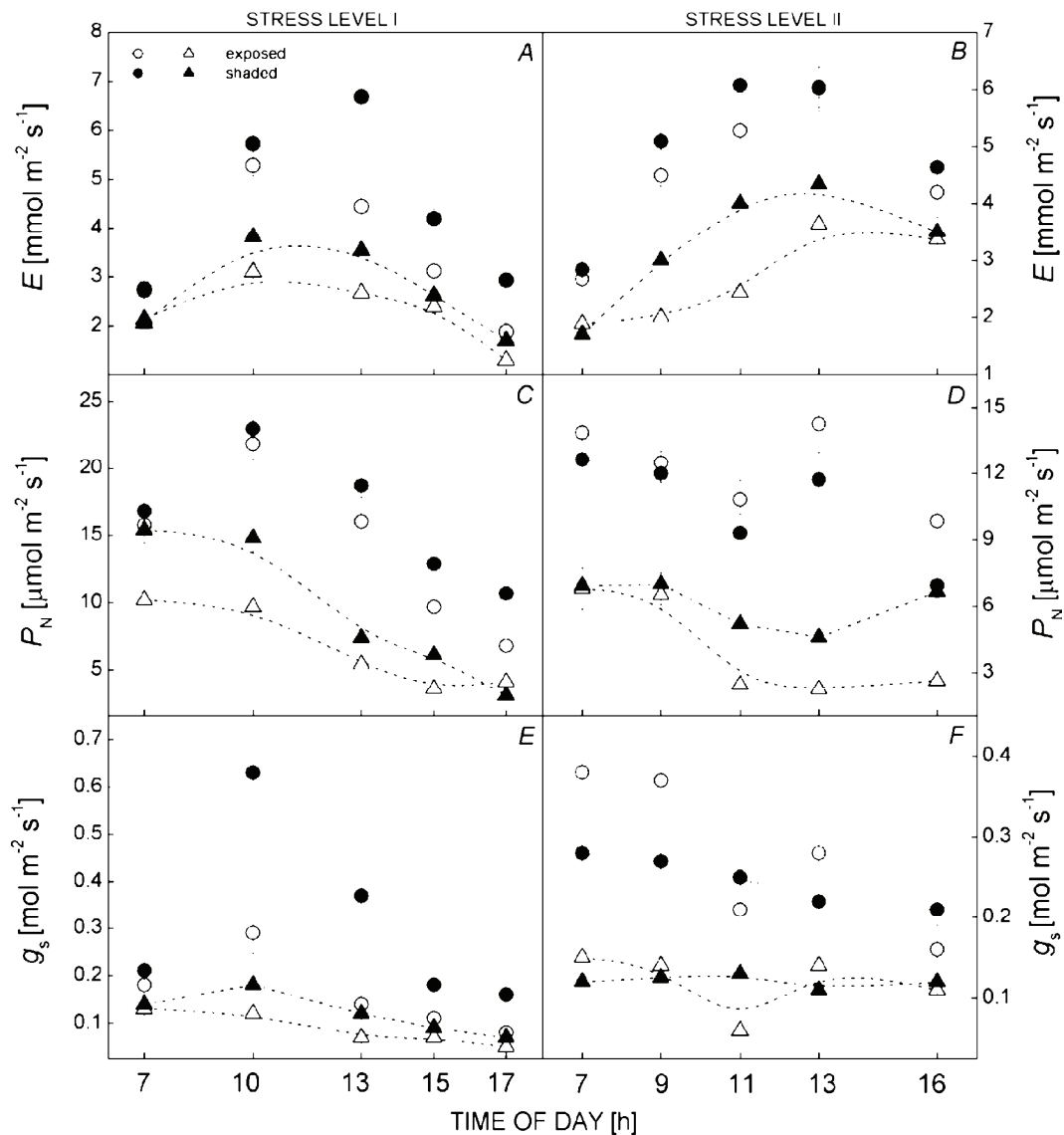


Fig. 4. Diurnal course of (A,B) transpiration E (C,D) net photosynthesis P_N and (E,F) stomatal conductance g_s of well-watered (circle, continuous line) and droughted (triangle, dashed line) leaves during the stress level I (left column) and II (right column). In all graphs (●, ▲) – shaded, (○, △) – exposed. Data are means \pm SE ($n = 16$), lines are illustrative only.

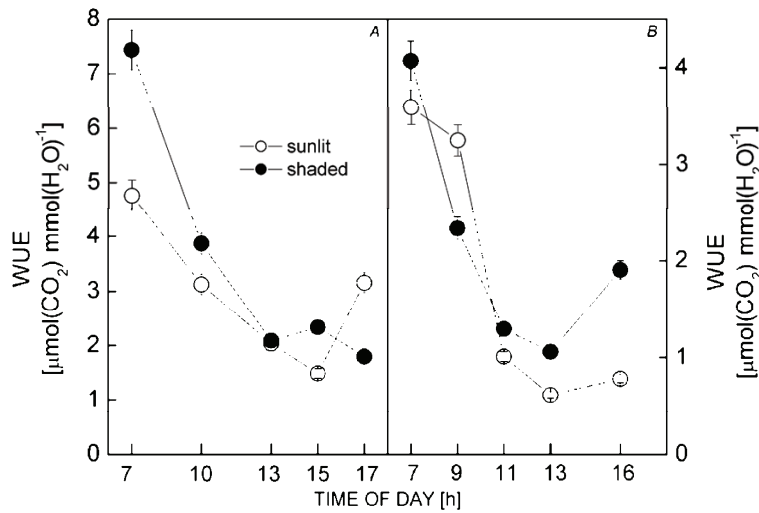


Fig. 5. Diurnal course of water-use efficiency (WUE) in sunlit (\circ) and shaded (\bullet) leaves of vines at (A) stress level I (-0.6 MPa pre-dawn Ψ_w) and (B) stress level II (-1.0 MPa pre-dawn Ψ_w). Data are means \pm SE ($n = 16$).

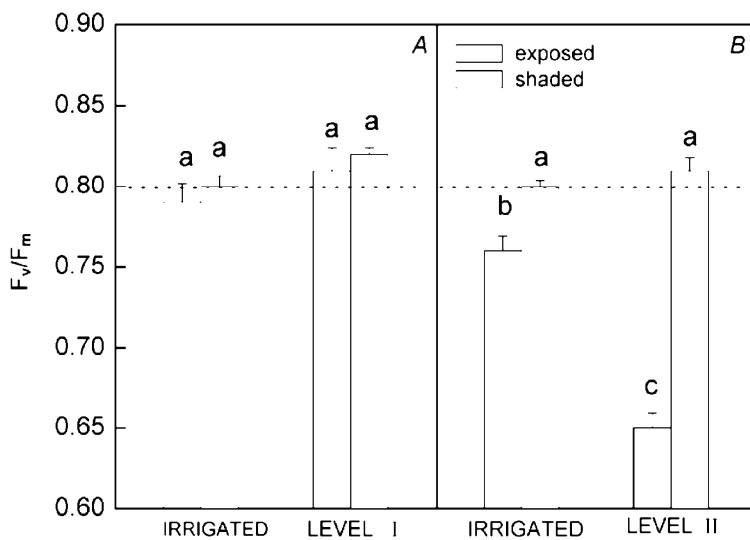


Fig. 6. Photochemical efficiency of photosystem II (F_v/F_m) (\pm SE) in naturally exposed (white column) and artificially shaded (grey column) leaves of irrigated and droughted vines. Measurements were taken between 13:00 and 14:00 h of (A) August 4 (stress level I) and (B) August 12 (stress level II). The horizontal dotted line posted at 0.8 represents the threshold below which photosystem was considered photoinhibited. Lowercase letters indicate statistically different means ($p < 0.05$) ($n = 16$).

After the drought cycle gradually developed over 25 days, leaf traits responded differently to drought and shade application. In D_{sun} leaves SLA noticeably increased (16–18 %) in both water stress levels (Table 1) according to findings in *Coriaria nepalensis* and *Vigna unguiculata* (Bargali and Tewari 2004, Anyia and Herzog 2004). That variation in SLA may indicate a higher positive carbon balance in these leaves presumably due to a decreased respiration rate under drought conditions (Flexas *et al.* 2006, Quero *et al.* 2006). The response of SLA to drought is not univocal and it is reported to be species-specific (Fernández and Reynolds 2000, Quero *et al.* 2006). Considering that lower SLA may confer drought tolerance in woody plants (Sack and Grubb, 2002), the increased SLA we found is in line with the high sensitivity of *Actinidia sp.* to water shortage (Judd *et al.* 1989).

Reduced radiation load did not significantly exert any pressure on SLA for all leaf types (Table 1) revealing that SLA in kiwifruit leaves was not sensitive to a 20-day shade period.

Concentrations of Chls in naturally exposed leaves did not significantly vary ($p < 0.05$) even after a 25-day drought period (Fig. 3) similarly to what was observed in apple and grapevine leaves (Šircely *et al.* 2005, Dobrowski *et al.* 2005). Although in some Mediterranean maquis species a decline of Chl content has been observed during drought (Gratani and Varone 2004). Hence, it seems that high-light grown leaves of *Actinidia deliciosa* do not strongly respond to drought adopting such a supplementary defence strategy (*i.e.* Chl content decrease) which reduced the possibility of further damage to the photosynthetic apparatus (Demmig-Adams and Adams 1992, Powles 1984). However, from the trend lines reported in Fig. 3, it could be interestingly inferred that deep drought synergistically acts with a low radiation increasing the abundance of Chls, even though variations were not statistically significant ($p < 0.05$). The higher Chl in shade tissues may be interpreted as an initial recovery from the full (excessive) light condition experienced during growth (Greer and Laing 1992). It is probable that a prolonged shade period could have a stronger impact on

Chl *a* and *b* contents as has already been reported for leaves of conifer species and *Phaseolus vulgaris* (Khan *et al.* 2004, Wentworth *et al.* 2006).

Photoinhibition and water-use efficiency: The maximum values of both P_N and g_s were recorded in all vines early in the morning when light was at saturating level intensities and VPD still relatively low. Thereafter, as PPFD and VPD rose net-photosynthesis decreased at midday as was previously observed (Buwalda *et al.* 1992, Gucci *et al.* 1996) (Figs. 1,4). Shade application considerably improved efficiency of water-use by droughted leaves when compared with those under full sunlight (Fig. 5). This is in accordance with findings in apricot (Nicolás *et al.* 2005), grape and citrus species (Jifon and Syvertsen 2003, Alarcón *et al.* 2006). Integrated daily WUE of D_{shade} leaves increased approximately by 20 % and 15 % in moderately and severely droughted vines respectively (Fig. 5). This was largely due to the increased photosynthesis rate induced by shade rather than the increment in transpiration (Fig. 4).

The interaction between the extent of applied water deficit and high irradiance drove the efficiency of photosynthetic energy conversion differently. The imposition of a mild drought did not provoke any drop of F_v/F_m in D_{sun} leaves (Fig. 6) indicating that stomatal limitations of photosynthesis predominated the metabolic limitations even though the maximum daily g_s was very low [*i.e.* $0.07 \text{ mol(H}_2\text{O)} \text{ m}^{-2} \text{ s}^{-1}$] (*see* Fig. 5). By contrast, in severely droughted vines experiencing full irradiance, photoinhibition was dramatically worsened by drought as

previously reported in Montanaro *et al.* (2007). It emerges that photoinhibition is the main limiting factor to carbon acquisition confirming the synergism between drought and high-light in relation to the inhibition of photosynthesis metabolism (Masojídek *et al.* 1991). Results proved that kiwifruit photosynthetic metabolism is quite resistant to drought and that it is progressively down-regulated as water stress intensifies according to the idea proposed by Flexas *et al.* (2004). Performance of the photosynthetic apparatus of shaded leaves was independent of soil water content and regularly optimal never being below 0.8 that is believed the threshold of photoinhibition (Krause and Weis 1991) (Fig. 6). Additionally, cloth application induced F_v/F_m in severely droughted vines to be approximately 25 % higher than those naturally exposed. A high F_v/F_m can result from an increase in F_m or a decrease in F_0 because F_v/F_m is derived as $(F_m - F_0)/F_m$ (Maxwell and Jonson 2000).

In conclusion, this study revealed that under a moderate stress level the photosynthetic performance is mainly regulated by stomata, while metabolic impairments of PSII limit carbon acquisition once vines are severely droughted. Results demonstrate that a 50 % reduction of PPFD over sun-acclimated leaves under drought increases carbon gain *via* reducing stomatal limitations and preventing inactivation of the primary photochemistry associated with PSII. This may represent a valuable intervention to save photosynthetic apparatus and increase WUE in vines experiencing typical Mediterranean summer stress.

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