# Response of photosynthetic machinery of field-grown kiwifruit under Mediterranean conditions during drought and re-watering

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## Abstract

Groups of *Actinidia deliciosa* A. Chev. C.F. Liang *et* A.R. Ferguson var. *deliciosa* kiwifruit plants were subjected to soil water shortage (D), while other groups were well irrigated (I). Variations in chlorophyll (Chl) *a* fluorescence indices and leaf gas exchange were determined once plants were severely stressed (25 d after the beginning of the D-cycle). Daily maximum values of photosynthetic photon flux density (PPFD) were *ca.* 1 650 μmol(photon) $m^{-2}$ $s^{-1}$, while air temperatures peaked at 34.6 °C. High irradiance *per se* did not greatly affect the efficiency of photosystem (PS) 2, but predisposed its synergistic reduction by D co-occurrence. Fluorescence showed transient photodamage of PS2 with a complete recovery in the afternoon in both D and I plants. Upon re-watering the efficiency of PS2 was suboptimal (95 %) at day 2 after irrigation was reinitiated. At early morning of the day 5 of re-watering, photosynthesis and stomatal conductance recovered at about 95 and 80 % of I vines, respectively, indicating some after-stress effect on stomatal aperture. Once excessive photons reached PS2, the thermal dissipation of surplus excitation energy was the main strategy to save the photosynthetic apparatus and to optimize carbon fixation. The rather prompt recovery of both Chl *a* fluorescence indices and net photosynthetic rate during re-watering indicated that kiwifruit photosynthetic apparatus is prepared to cope with temporary water shortage under Mediterranean-type-climates.

*Additional key words*: *Actinidia*; chlorophyll content; drought relief; excessive irradiation; fluorescence; net photosynthetic rate; photoinhibition; semi-arid conditions; stomatal conductance; transpiration rate.

## Introduction

Irradiation and water are main resources affecting leaf traits and regulating plant growth and survival. Photon energy is the key driving force for photosynthesis, however, above the photosynthetic saturation excessive irradiance may be the key limiting factor reducing photosystem (PS) 2 efficiency (*i.e.* photoinhibition; Demmig-Adams and Adams 1992). The Mediterranean region is characterised by dry summers, with high temperatures and little or no precipitation, consequently irrigation is typically used to compensate for inadequate rainfall for most cultivated plants, avoiding restriction of physiological processes induced by water stress (Flexas *et al.* 1999). However, long-term models predict a decrease of natural water resources for the Mediterranean agriculture as a consequence of changing climate (Katerrji *et al.* 2006). Thus, plants (especially those sensitive to water shortage) could suffer from the combination of drought with other typical climatic parameters (*i.e.* high irradiance, elevated air temperatures, and high leaf-to-air vapour pressure deficit, VPD).

The response of photosynthetic apparatus to the interactions of drought, high irradiance, and temperatures have been extensively studied for species deriving from *in situ* evolution typically adapted to Mediterranean-type climates (among others *Olea europaea*, *Quercus spp.*, *Arbutus unedo, Rosmarinus officinalis*—Angelopoulos *et al.* 1996, Gratani and Varone 2004, Quero *et al.* 2006) revealing that the effects of low water status on the susceptibility of PS2 to photodamage are species-specific (Valladares and Pearcy 1997). However, for some non-endemic Mediterranean species, such effects have not been explored adequately. This is the case with kiwifruit (*Actinidia deliciosa* var. *deliciosa*).

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*Abbreviations*: Chl – chlorophyll; D – drought; $E$ – leaf transpiration rate; $F_m$ – maximum fluorescence of dark-adapted leaves; $F_0$ – minimum fluorescence of dark-adapted state; $g_s$ – stomatal conductance; I – irrigated; NPQ – non-photochemical quenching of variable fluorescence; $P_N$ – net photosynthetic rate; PPFD – photosynthetic photon flux density; PS2 – photosystem 2; SLA – specific leaf area; VPD – leaf-to-air vapour pressure deficit; $\Psi_{PDleaf}$ – predawn leaf water potential.
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High leaf tissue density, leaf thickness, and reduced leaf area are traits improving drought resistance by decreasing photochemical damage to the photosynthetic system (Gratani and Varone 2004). Leaves of kiwifruit have no conspicuous photoprotective morphological characteristics potentially making them highly susceptible to both photodamage and drought (Greer and Laing 1992, Gucci *et al.* 1996). This is possibly because of the ecophysiology of the *Actinidia* species which originate from habitats characterised by high humidity and only a moderate intensity of sunlight as reviewed by Ferguson (1984).

The propensity for photoinhibition in field-grown kiwifruit has been studied mainly at maximum air temperatures of 18–25 °C showing that in spite of high irradiance [PPFD, Photosynthetic Photon Flux Density of 1 700 μmol(photon) $m^{-2}$ $s^{-1}$] there was no specific evidence of diurnal high-irradiance-induced photoinhibition or for increased non-radiative energy dissipation (Greer and Laing 1992, Greer 1995). In the Mediterranean during a summer day, air temperature frequently rises up to 35 °C, moreover solar radiation may easily reach 1 800–2 000 μmol(photon) $m^{-2}$ $s^{-1}$ PPFD for several hours, that is well beyond the photosynthetic saturation irradiance for kiwifruit (Greer and Halligan 2001). The interaction between excessive irradiance and high temperatures induces photoinhibition (Powles 1984), therefore it is reasonable to hypothesise that under the combination of high air temperatures and excessive radiation (a very common event in Mediterranean) kiwifruit suffers photoinhibition, which is aggravated by reduced soil water content.

Stomatal regulation of leaf gas exchange is a key-factor for the survival of drought-tolerant species even though this reduces plant carbon gain (Dichio *et al.* 2006). However, whether drought mainly limits photosynthesis through stomatal closure or by metabolic impairment is still controversially debated (Flexas and Medrano 2002). In kiwifruit, the effectiveness of leaf photosynthetic activity control by stomata is unclear. The afternoon depression of photosynthesis in field-grown well-watered vines is closely correlated with stomatal closure and stomata are highly sensitive to changes in soil water status (Buwalda *et al.* 1992, Gucci *et al.* 1996). However, leaves are unable to regulate stomatal conductance ($g_s$) with increasing soil water deficit, thus leaves under that condition continue to lose water, which is likely to increase photoinhibition severity (Judd *et al.* 1986a, Medina *et al.* 2002). There are still open issues to investigate the response of kiwifruit photosynthetic machinery to different light–water scenarios, in order to further characterise the physiology of kiwifruit in view of the changing global environment.

Therefore, the main objective of this study was to ascertain the acclimation capacity of kiwifruit to the multiple co-occurring summer stresses (high irradiance and temperature) combined with drought, that is likely to be more frequently experienced in the Mediterranean region (Katerrji *et al.* 2006). Upon re-watering, vegetative growth of stressed plants can recover mainly in relation to the degree of previous water stress reached (Flexas *et al.* 2004), indicating a reversibility of morphological and physiological changes promoted by drought.

Till now, in field-grown kiwifruit that simultaneously experienced high irradiance + temperature and drought, the efficiency of PS2 during re-watering has not been adequately studied. This is why we studied the responses of photosynthesis to water stress and during re-watering in kiwifruit vines grown in a Mediterranean site, both in terms of $CO_2$ assimilation and functionality of the photosynthetic apparatus, as assessed by chlorophyll (Chl) *a* fluorescence measurements. Changes of Chl content and Specific Leaf Area (SLA) were also examined.

## Materials and methods

**Experimental design**: Trials were carried out in Southern Italy at the "Pantanello" Agricultural Experimental Station near Metaponto (N40°23', E16°46') on mature own-rooted field-grown kiwifruit vines (*Actinidia deliciosa* A. Chev. C.F. Liang *et* A.R. Ferguson var. *deliciosa*, cv. Hayward) during the summer of 2004. Plants were trained onto Pergolas at 494 plants per ha, with N-S row orientation. During the experiment, control vines were regularly microjet-irrigated (wetting the whole soil surface area), approximately every 4–5 d on evapotranspirative demand basis, and so as to maintain soil moisture levels uniformly at around 90 % of field capacity (I-plants).

From July 17$^{th}$ on, progressive soil-water depletion was applied by withholding irrigation to 20 vines distributed within three rows. Measurements were performed on 12 August (*i.e.* 25 d after withholding irrigation) to study severely stressed vines, whose water status was defined on the basis of recordings of predawn leaf water potential (see below). In the late afternoon (~19:30 h) of that last day of water stress cycle (day 0), drought (D) was relieved by re-watering the vines to soil capacity and recovery was followed until day 13.

**Environment and plant water status**: Irradiance was measured using 3 quantum sensors (model *SKP 215*, *Skye Instruments*, Llandrindod Wells, UK). The sensors were placed 2 m above the canopy to measure the incident PPFD. Temperature and humidity were also monitored using 3 sensors (model *HUMITER 50Y, Vaisala,* Helsinki, Finland). These were disposed close the irradiance sensors. All sensors were connected to a datalogger (*CR10*, *Campbell Scientific*, USA), which was programmed to record at 60-s intervals and to compute and store averages at 15-min intervals. The leaf-to-air VPD was calculated from air temperature and relative

humidity 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* model *6050X1*, *Soil Moisture Equip. Corp.*, USA) every 2–3 d. Measurements were always performed at midday (11:00–12:00 h). Moisture levels (v/v) in irrigated (I) and droughted (D) soils are the averages of measurements at three points.

Plant water status was assessed through predawn leaf water potential ($\Psi_{PDleaf}$) which was measured between 03:30 and 04:30 h, on three leaves per plant (similar to those used for gas exchange analyses) using a Scholander pressure chamber (*PMS Instrument*, Corvallis, OR, USA) pressurized with nitrogen according to the procedure recommended by Turner (1981).

**Leaf gas exchange** was measured once plants were severely water-stressed (*i.e.* $\Psi_{PDleaf}$ close to –1.0 MPa). Net photosynthetic rate ($P_N$), transpiration rate ($E$), and $g_s$ were measured using a portable gas exchange system *ADC-LCA4* operating at a flow rate of 200 μmol $s^{-1}$ under the prevailing environmental condition. The ADC system was equipped with a *PLC4B* chamber (*ADC*, 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. Measurements were carried twice a day (*i.e.* early in the morning at 07:00 h and at midday) always on the same leaves. 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 on the same leaf used for gas exchange measurements (from 08:00 to 19:00 h every three hours during 12 August, early in the morning and at midday – between 13:30 and 14:00 during the recovery) using a pulse amplitude modulation fluorometer *PAM-2000* (*H. Walz*, Effeltrich, Germany) connected to a personal computer with data acquisition software *DA-2000*. Measurements of the minimal ($F_0$) and maximal ($F_m$) fluorescence were made from leaves maintained in the dark for about 30 min using the dark leaf clip, the latter was achieved by applying a brief saturating pulse (5 000 μmol $m^{-2}$ $s^{-1}$). The maximal photochemical efficiency of PS2 ($F_v/F_m$), where $F_v = F_m - F_0$, was then calculated. The non-photochemical quenching (NPQ) was determined according to Maxwell and Johnson (2000).

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 content** was determined in three discs (25 mm diameter) on 3 leaves per plant (3× treatment) sampled at the end of the measurement day. After centrifugation of 80 % acetone extracts, absorbances of supernatant at 625, 647, and 664 nm were spectrophotometrically determined (*Varian AA-40*) and the amount of Chl was calculated by the formulae reported by Moran (1982). On the same leaves, two discs were sampled and dried to constant mass (48 h at 60 °C, ventilated oven) to determine SLA [$cm^2$ $kg^{-1}$].

**Descriptive statistics** and curve fitting were made by *Origin®6.1* (*OriginLab Corporation*, USA).

## Results

**Environmental conditions and leaf traits**: Soil water content (not shown) gradually decreased in the areas subjected to water restriction reaching the minimum value of *ca*. 25 % (v/v) after 24 d while in the I-plants it was on average close to 38 %. In response to progressive soil-water depletion, the predawn leaf water potential, $\Psi_{PDleaf}$ (not shown), at 18 and 25 d after water was withheld, reached –0.65 and –1.00 MPa, respectively.

Irradiance, air temperature, and air humidity were typical of Mediterranean summer conditions. Data taken on the measurement day (25 d after water was withheld) show (Fig. 1) that global radiation over the canopy reached its maximum of 1 650 μmol(photon) $m^{-2}$ $s^{-1}$ in PPFD at midday (11:00–14:00 h), in addition air temperature peaked at 34.6 °C (Table 1). In parallel, the VPD reached its maximum at midday (*i.e.* 4.5 kPa, Fig. 1). Only a few leaves (<5 % per plant) of D-plants exhibited visible symptoms of injury (*e.g.* necrotic spots); however, those utilised for all determinations were undamaged.

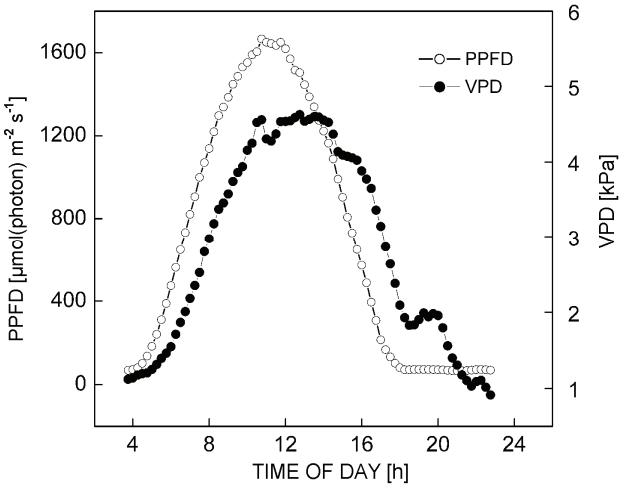


Fig. 1. Diurnal course of irradiance (PPFD) and vapour pressure deficit (VPD) during the last day of drought cycle (*i.e.* 12 August).

Table 1. Air and adaxial leaf temperatures [°C] (±SE) measured early in the morning (07:00 h) and at midday (13:00 h) on the last day of the drought cycle and during re-watering in irrigated (I) and water-stressed (D) vines. Within a column, values followed by different letters are significantly different, $p$=0.05 (Student's $t$-test).

| | Last (0) day of drought cycle | | Day of re-watering (always at 13:00 h) | | | |
|---|---|---|---|---|---|---|
| | 07:00 h | 13:00 h | 1st | 2nd | 5th | 13th |
| Air | 27.2 ± 0.5 a | 34.6 ± 0.4 a | 33.2 ± 0.2 a | 32.9 ± 0.2 a | 34.9 ± 0.1 a | 32.1 ± 0.1 a |
| I-leaves | 28.7 ± 0.5 a | 36.5 ± 0.7 a | 35.0 ± 0.2 b | 35.4 ± 0.3 b | 37.5 ± 0.5 b | 34.1 ± 0.2 b |
| D-leaves | 30.3 ± 0.4 b | 39.7 ± 0.5 b | 36.5 ± 0.3 b | 35.8 ± 0.4 b | 37.7 ± 0.9 b | 34.7 ± 0.1 b |

Table 2. Chlorophyll (Chl) contents and specific leaf area (SLA) in fully expanded leaves of kiwifruit plants as affected by different water status (I – irrigated, D – drought-stressed). *means significant difference in column at $p$<0.05 by Student's $t$-test.

| | Chl *a* [mg m$^{-2}$] | Chl *b* | Chl *a*/*b* | SLA [m$^2$ kg$^{-1}$] |
|---|---|---|---|---|
| I | 233.58 | 71.51 | 3.26 | 13.3 |
| D | 211.75 | 71.96 | 2.97 | 15.4* |

In I-vines, leaf temperature at 13:00 h significantly increased by approximately 7 °C compared to the values recorded early in the morning, and surprisingly in D-leaves it rose to 39 °C, *i.e.* increased by about 10 °C (Table 1).

Detailed analyses in fully expanded leaves revealed that Chl content remained unaffected by D application, while SLA significantly increased ($p$=0.05, Student's $t$-test) in D-vines (Table 2).

**Gas exchange and fluorescence**: $P_N$ was higher early in the morning during sunshine (*i.e.* at 07:00 h) being *ca*. 16 and 7 μmol($CO_2$) m$^{-2}$ s$^{-1}$ in I- and D-plants, respectively (Table 3). At midday, as leaf water potential dropped (not shown), even though PPFD reached its maximum value, a marked depression in photosynthetic activity occurred in both treatments. $P_N$ declined to approximately 11 μmol($CO_2$) m$^{-2}$ s$^{-1}$ in I-plants, while in the D-plants it fell to 2.4 μmol($CO_2$) m$^{-2}$ s$^{-1}$ (Table 3). $g_s$ declined at midday in both treatments, reaching 0.21 and 0.06 mol($H_2O$) m$^{-2}$ s$^{-1}$ in I- and D-plants, respectively (Table 3). Early in the morning, the intrinsic photosynthetic capacity ($P_N/g_s$) was slightly higher (approximately 10 %) in D-plants. At midday that parameter showed an opposite behaviour. In I-vines it increased by about 23 % and conversely it showed a 10 % drop off in D-plants (Table 3).

As expected, $E$ was strongly affected by PPFD and VPD exhibiting low values early in the morning and reaching higher values at midday correspondingly to highest PPFD and VPD (Table 3, Fig. 1).

Table 3. Changes in net-photosynthetic rate ($P_N$) [μmol($CO_2$) m$^{-2}$ s$^{-1}$], stomatal conductance ($g_s$) [mol($H_2O$) m$^{-2}$ s$^{-1}$], transpiration rate ($E$) [mmol($H_2O$) m$^{-2}$ s$^{-1}$], and intrinsic water-use efficiency ($P_N/g_s$) [μmol($CO_2$) mol$^{-1}$($H_2O$)] measured early in the morning (07:00 h) and at midday (13:00 h) in leaves of irrigated (I) and severely water-stressed (D) vines. Means ± SE from at least sixteen measurements of three independent plants.

| | Early morning | | | | Midday | | | |
|---|---|---|---|---|---|---|---|---|
| | $P_N$ | $g_s$ | $E$ | $P_N/g_s$ | $P_N$ | $g_s$ | $E$ | $P_N/g_s$ |
| I | 15.88±1.07 | 0.38±0.04 | 2.67±0.19 | 41.78±1.30 | 10.82±2.11 | 0.21±0.03 | 5.28±0.47 | 51.50±2.10 |
| D | 6.79±0.94 | 0.15±0.02 | 1.89±0.21 | 45.26±1.90 | 2.45±0.47 | 0.06±0.00 | 2.43±0.14 | 40.83±1.80 |

The diurnal variations in fluorescence indices $F_m$, $F_v$, and $F_v/F_m$ clearly declined in all treatments to minimum values at 14:00 h and increased again in the afternoon, concomitantly $F_0$ exhibited an opposite behaviour rising to the maximum (Fig. 2). In any case, the fluorescence indices oscillated corresponding to rising VPD and PPFD through the day.

In irrigated plants, the extent of the midday fall of $F_v$ and $F_m$ was 22 and 16 % of the early morning value, respectively. The depressions of $F_v$ and $F_m$ were more pronounced in D-plants, reaching 47 ($F_v$) and 38 ($F_m$) % of the early morning values resulting in a pronounced decrease in $F_v$ /$F_m$ during the hottest hours of the day (Fig. 2*D*). Essentially, the minimal Chl *a* fluorescence showed an opposite trend increasing in both treatments by about 13–16 % to a maximum value at midday and declining in the afternoon, in addition already early in the morning D-leaves exhibited $F_0$ to be 20 % higher than in irrigated ones (Fig. 2*A*).

Generally, leaves exhibited higher non-photochemical quenching, NPQ early in the morning, but this was sustained in D-leaves till 14:00 h, while it sharply declined to approximately 30 % at 11:00 h in I-leaves (Fig. 3). Afterwards, NPQ progressively declined to a minimum value in the late afternoon concurrently with the decrease in radiant energy availability (Fig. 3).

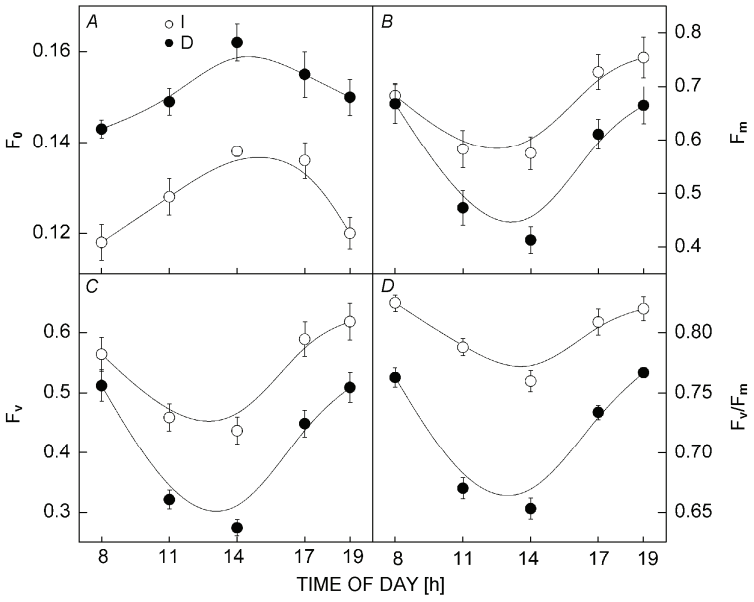


Fig. 2. Diurnal course of the minimal, $F_0$ (*A*), maximal, $F_m$ (*B*), and variable, $F_v$ (*C*) fluorescence and the $F_v/F_m$ ratio (*D*), in irrigated, I (○) and water-stressed, D (●) leaves during the day of severe stress. *Bars* are standard errors, *lines* are illustrative only.

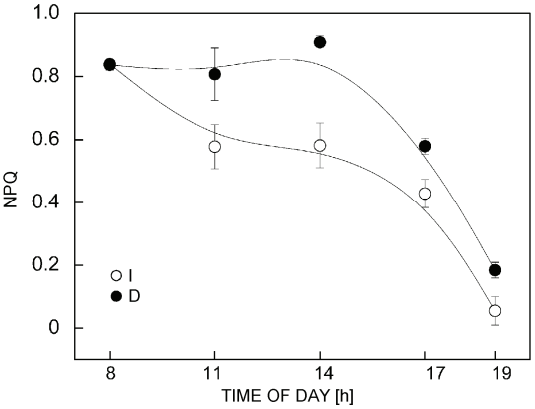


Fig. 3. Diurnal course of non-photochemical quenching (NPQ) in leaves of irrigated, I (○) and water-stressed, D (●) plants during the last day of drought cycle. *Bars* are standard errors, *lines* are illustrative only.

**Recovery of photosynthetic activity upon re-watering**: By day 1 after reinitiating the irrigation the $\Psi_{PDleaf}$ of D-plants rapidly recovered (not shown) to values equal to those of I-plants (at about –0.2 MPa). Furthermore, leaf temperatures recorded at midday were similar between the treatments ranging from 34.1 to 37.7 °C within the recovery period (Table 1). $P_N$ recovered to values up to 80 % of I-plants by day 2 after re-watering with slight differences between values recorded early in the morning and at midday (Fig. 4*A*). $g_s$ of these plants showed a slow rate of recovery particularly during the hottest hours of the day, being equal to 60 % of I-plants even 13 d after irrigation was reinitiated (Fig. 4*B*). The photochemical efficiency of PS2 (*i.e.* $F_v/F_m$) rapidly recovered being only just below the values for I-plants by day 2 after re-watering (Fig. 5*A*). Recovery of plant water status steeply lowered the non-radiative energy dissipation (NPQ) of D-plants towards the I-plant values. Moreover, it was approximately 20 % lower at noon of day 2 (Fig. 5*B*). However, both $F_v/F_m$ and NPQ did not completely recover (94 %) till 13 d after plant water status was again optimal.

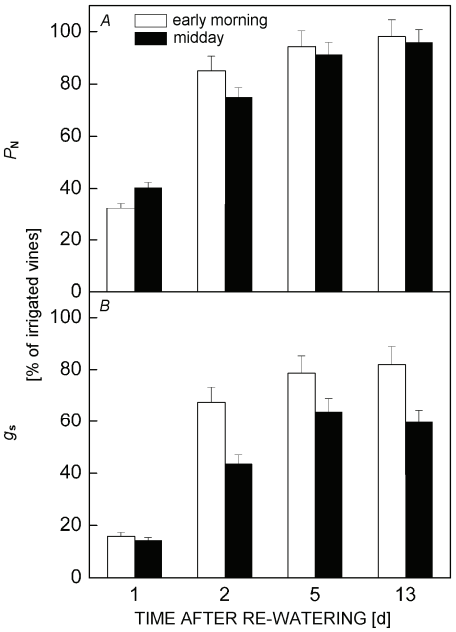


Fig. 4. Net-photosynthetic rate, $P_N$ (*A*) and stomatal conductance, $g_s$ (*B*) of water-stressed plants as % of irrigated plants during re-watering. Measurements were taken at early morning (07:00 h) and midday (13:00 h). Means ± SE of at least sixteen measurements on three plants.

## Discussion

In this study, plants gradually experienced water shortage, being stressed only 25 d after the beginning of the D-cycle. Based on predawn leaf water potential reached (*i.e.* –1.0 MPa), plants were defined "severely" stressed according to Gucci *et al.* (1996). Such a slow application of D reasonably mimics the occurrence of a dry period in an open field, allowing osmotic and other physiological adjustments of leaf.

The leaf traits we considered (*i.e.* SLA and Chl) showed a different response to D application. The SLA was noticeably higher (17 %) in leaves subjected to D as compared to I-leaves, which is similar to the findings of Bargali and Tewari (2004) for *Coriaria nepalensis.* It may indicate a higher positive carbon balance in these leaves (Quero *et al.* 2006) presumably due to decreased respiration rate under severe D (Flexas *et al.* 2006). The reverse is true for Chl content, that remained substantially insensitive to D-application similarly to what was observed in apple and grapevine leaves (Dobrowski *et al.* 2005, Šircely *et al.* 2005), although in some Mediterranean maquis species a decline of Chl content has been observed during D (Gratani and Varone 2004). Hence, *A. deliciosa* is probably not able to adopt that supplementary defence strategy (*i.e.* Chl content decrease) which reduces the possibility of further damage to the photosynthetic apparatus (Demmig-Adams and Adams 1992, Powles 1984).

The higher values of both $P_N$ and $g_s$ were recorded in all vines early in the morning when irradiance was saturating and VPD still relatively low. Thereafter, as PPFD and VPD rose, $P_N$ decreased at midday as already observed before now (Buwalda *et al.* 1992, Gucci *et al.* 1996). The analysis of metabolic limitation of photosynthesis through the intrinsic-water-use efficiency (*i.e.* $P_N/g_s$) may be indicative of the status of PS2. In irrigated plants, the $P_N/g_s$ increased at midday (Table 3) suggesting that stomatal closure is still the dominant limitation to photosynthesis, while the decrease of $P_N/g_s$ (Table 3) revealed the effect of D in combination with high irradiance and temperature upon non-stomatal limitation of photosynthesis (Flexas *et al.* 2004). The behaviour of $g_s$ further supports that conclusion. In D-plants $g_s$ decreased dramatically at midday to 0.06 mol($H_2O$) $m^{-2}$ $s^{-1}$ (see Table 3) being very close to the threshold of 0.05 mol($H_2O$) $m^{-2}$ $s^{-1}$ that generally identifies the stage at which metabolic limitations of photosynthesis occur (Flexas *et al.* 2004).

At the end of the D-cycle, the photochemical efficiency of PS2 (assessed by $F_v/F_m$) exhibited an unequivocal response to concomitantly high irradiance + temperature and water deficit. At midday $F_v/F_m$ considerably decreased starting at 0.79 early in the morning and falling towards 0.60. In irrigated vines, there was a slight midday oscillation of $F_v/F_m$ which decreased to 0.76 remaining very close to the threshold of photoinhibition (*i.e.* 0.8; cf. Krause and Weis 1991) (Fig. 2*B*).

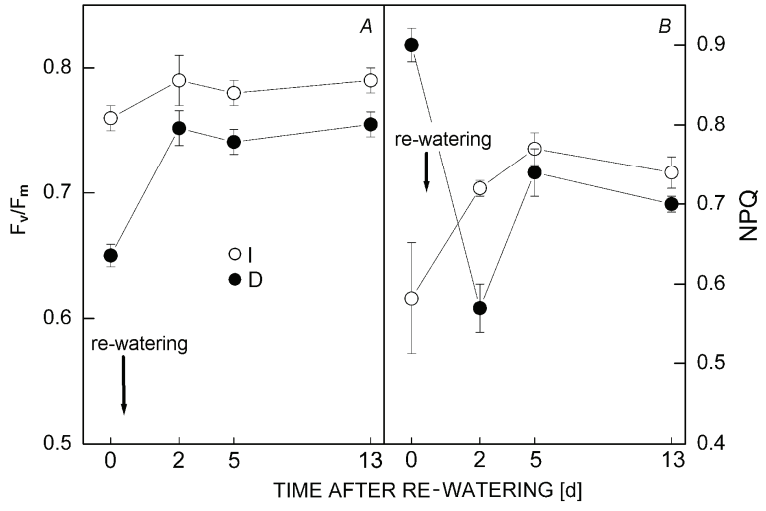


Fig. 5. Behaviour of photochemical efficiency of PS2 ($F_v/F_m$) and of non-photochemical quenching (NPQ) in irrigated (I) and droughted (D) plants during re-watering. Means ± SE of sixteen measurements recorded between 13:30 and 14:00 h; day 0 is the last day of the D-cycle.

However, in spite of the narrow $F_v/F_m$ oscillation (about 8 %) the wide midday fluctuations of $F_0$ and $F_m$ (around 17 %) in I-leaves (Fig. 2) suggest that PS2 was partially inactivated during the hottest hours of the day (*i.e.* air temperature up to 34 °C, see Table 1). This is evidence that typical Mediterranean summer stresses may limit the photosynthetic energy conversion in kiwifruit although plants are well-watered which partially contrasts with previous findings. Greer (1995) found no significant diurnal changes in fluorescence indices in a field experiment. Greer and Laing (1992) reported that kiwifruit leaves are susceptible to photoinhibition only during spring and autumn. This apparent disagreement may be explained considering that the relatively mild environment during their experiments (maximum air temperature of 18–25 °C) probably masked the susceptibility of

kiwifruit leaves to photoinhibition.

A midday decrease in $F_v/F_m$ and subsequent recovery in the afternoon may reflect a regulatory response of the photosynthetic system to excess absorbed photon energy (Werner *et al*. 1999). Our data show that "dynamic" photoinhibition also occurs in kiwifruit under Mediterranean summer when exacerbated by severe D, representing a key survival-factor for *A. deliciosa*.

The abrupt fall of $F_v/F_m$ in D-leaves detected at midday was in part due to the sudden increase of the minimal Chl *a* fluorescence ($F_0$, see Fig. 2*A*), which was probably due to inhibition of the acceptor side of PS2 (Bertamini and Nedunchezhian 2003). The highest $F_0$ recorded early in the morning in D-vines (about 20 % higher than that in I-plants), may well be the response to prolonged and gradual soil water shortage due to the progressively depression of transcription of several genes associated with the structure and function of photosynthesis (Flexas *et al.* 2006). In similar experimental conditions (Angelopoulos *et al.* 1996), leaves of potted olive trees after a 10-d D-cycle were severely stressed (*ca*. –6 MPa $\Psi_{PDleaf}$), but showed early morning $F_0$ comparable to that of I-vines. This difference between olive and kiwifruit underlines that endemic and non-endemic Mediterranean species respond differently to D presumably because their morpho-anatomical and physiological traits result in a different adaptive photosynthetic strategy (Ferguson 1984, Gratani and Varone 2004).

The sustained non-photochemical quenching (NPQ) in D-plants supports the interpretation that the sudden decline in $F_v/F_m$ is mainly due to an increase in protective non-radiative energy dissipation. Leaf temperature further corroborates this idea. It peaked up to 39 °C being 5 °C higher than air temperature according to other studies (Buwalda *et al.* 1992, Allan and Carlson 2003). The temperature at which PS2 is thermally denatured ranges from 43 to 49 °C (Terzaghi *et al.* 1990); hence it was assumed that temperatures reached by leaves in this experiment (always below 40 °C) did not cause thermal damage.

In our study non-photochemical quenching vanished in the late afternoon when irradiance decreased as predicted by Demmig-Adams and Adams (1992), moreover, it was greater in D-plants (Fig. 3). To explain the origin of a sustained NPQ both the change in the bulk of xanthophyll pool components and a persistent de-epoxidation state of the xanthophyll cycle pigments has been proposed (Valladares and Pearcy 1997), although in cells of *Chlorella vulgaris* antheraxanthin formed during the epoxidation reaction was able to quench Chl fluorescence non-photochemically (Goss *et al.* 2006). For kiwifruit leaves, the involvement of the xanthophyll cycle for photoprotection capacity has been demonstrated by Greer *et al.* (1993).

Pre-dawn leaf water potential promptly recovered at values of I-vines (not shown) on day 1 after irrigation was reinitiated according to previous observations on kiwifruit (Judd *et al.* 1986b, Gucci *et al.* 1996). Upon the relief from water deficit a rapid recovery of $P_N$ was detected at day 5 (85 %) which was very close to be completely restored (98 %) a week later (Fig. 4*A*). The rate of recovery of $P_N$ was rather slow compared to that of vines subjected to a mild stress (–0.40 MPa $\Psi_{PDleaf}$) (Gucci *et al.* 1996) according to the idea that the rate of recovery depends on the level of stress previously reached (Flexas *et al*. 2004). The Fig. 4*B* shows an after-stress effect on stomata, whose midday conductance partially recovered being no more than 60 % of irrigated vines, although this delay did not limit $P_N$. The D-induced alteration in bulk of abscisic acid may explain the slow stomatal recovery, that could be a result of the persistent effects of that hormone produced during the water stress period (Miller *et al.* 1998) strengthened by variations in leaf apoplastic and root sap pH (Davies *et al.* 2000). The recovery of $g_s$ (although partial) contributed to re-establish the thermoregulatory capacity of leaf reducing heat stress (see midday leaf temperatures in Table 1).

On the second day of re-watering, the efficiency of PS2 (*i.e*. $F_v/F_m$) quickly recovered at values close to those of well-watered vines (95 %), remaining stable till the end of experiment (*i.e*. 13 d after re-watering) (Fig. 5*A*). The non-radiative energy dissipation showed a parallel behaviour during re-watering (Fig. 5*B*).

This study reveals that *A. deliciosa* deals with the Mediterranean environment through the combination of low $g_s$ and enhanced NPQ, *i.e*. it sacrifices carbon gain for water conservation and photoprotection (*via* energy dissipation). Moreover, our results demonstrated that this strategy is also actuated under severe D. Considering the rather prompt recovery of both $P_N$ and PS2 efficiency once plant water status was again optimal, we conclude that kiwifruit photosynthetic apparatus is prepared to face a temporary water shortage, which is predicted in the future to be even more frequently experienced in Mediterranean-type regions (Katerrji *et al*. 2006).

## References

Allan, P., Carlson, C.: Effects of shade level on kiwifruit leaves efficiency in a marginal area. – Acta Hort. **610**: 509-516, 2003.

Angelopoulos, K., Dichio, B., Xiloyannis, C.: Inhibition of photosynthesis in olive trees (*Olea europaea* L.) during water stress and rewatering. – J. exp. Bot. **47**: 1093-1100, 1996.

Bargali, K., Tewari, A.: Growth and water relation parameters in drought-stressed *Coriaria nepalensis* seedlings. – J. Arid Environ. **58**: 505-512, 2004.

Bertamini, M., Nedunchezhian, N.: Photoinhibition of photosynthesis in mature and young leaves of grapevine (*Vitis vinifera* L.). – Plant Sci. **164**: 635-644, 2003.

Buwalda, J.G., Green, T.G.A., Curtis, J.P.: Canopy photosynthesis and respiration of kiwifruit (*Actinidia deliciosa* var. *deliciosa*) vines growing in the field. – Tree Physiol. **10**: 327-341, 1992.

Davies, W.J., Bacon, M.A., Thompson, D.S., Sobeih, W., González Rodríguez, L.: Regulation of leaf and fruit growth in plants growing in drying soil: exploitation of the plants' chemical signalling and hydraulic architecture to increase the efficiency of water use in agriculture. – J. exp. Bot. **51**: 1617-1626, 2000.

Demmig-Adams, B., Adams, W.W., III: Photoprotection and other responses of plants to high light stress. – Annu. Rev. Plant Physiol. Plant mol. Biol. **43**: 599-626, 1992.

Dichio, B., Xiloyannis, C., Sofo, A., Montanaro, G.: Osmotic regulation in leaves and roots of olive trees during a water deficit and rewatering. – Tree Physiol. **26**: 179-185, 2006.

Dobrowski, S.Z., Pshnik, J.C., Zarco-Tejada, P.J., Ustin, S.L.: Simple reflectance indices track heat and water stress-induced changes in steady-state chlorophyll fluorescence at the canopy scale. – Remote Sens. Environ. **97**: 403-414, 2005.

Ferguson, A.R.: Kiwifruit a botanical review. – Hort. Rev. **6**: 1-64, 1984.

Flexas, J., Bota, J., Cifre, J., Escalona, J.M., Galmés, J., Gulías, J., Lefi, E., Martínez-Cañellas, S.F., Moreno, M.T., Ribas-Carbó, M., Riera, B., Sampol, D., Medrano, H.: Understanding down-regulation of photosynthesis under water stress future prospects and searching for physiological tools for irrigation management. – Ann. appl. Biol. **144**: 273-283, 2004.

Flexas, J., Bota, J., Galmés, J., Medrano, H., Ribas-Carbó, M.: Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. – Physiol. Plant. **127**: 343-352, 2006.

Flexas, J., Escalona, J.M., Medrano, H.: Water stress induces different levels of photosynthesis and electron transport rate regulation in grapes. – Plant Cell Environ. **22**: 39-48, 1999.

Flexas, J., Medrano, H.: Drought-inhibition of photosynthesis in $C_3$ plants stomatal and non-stomatal limitations revisited. – Ann. Bot. **89**: 183-189, 2002.

Goss, R., Lepetit, B., Wilhelm, C.: Evidence for a rebinding of antheraxanthin to the light-harvesting complex during the epoxidation reaction of the violaxanthin cycle. – J. Plant Physiol. **163:** 585-590, 2006.

Goudriaan, J., van Laar, H.H.: Modelling Potential Crop Growth Processes. – Kluwer Academic Publ., Dordrecht – Boston – London 1994.

Gratani, L., Varone, L.: Adaptive photosynthetic strategies of the Mediterranean maquis species according to their origin. – Photosynthetica **42**: 551-558, 2004.

Greer, D.H.: Effect of canopy position on the susceptibility of kiwifruit (*Actinidia deliciosa*) leaves on vines in an orchard environment to photoinhibition throughout the growing season. – Aust. J. Plant Physiol. **22**: 299-309, 1995.

Greer, D.H., Halligan, E.A.: Photosynthetic and fluorescence light response for kiwifruit (*Actinidia deliciosa*) leaves for different stages of development on vines grown at two different photon flux densities. – Aust. J. Plant Physiol. **28**: 373-382, 2001.

Greer, D.H., Laing, W.A.: Photoinhibition of photosynthesis in intact kiwifruit (*Actinidia deliciosa*) leaves changes in susceptibility during the growth season. – Planta **186**: 418-425, 1992.

Greer, D.H., Laing, W.A., Woolley, D.J.: The effect of chloramphenicol on photoinhibition of photosynthesis and its recovery in intact kiwifruit (*Actinidia deliciosa*) leaves. – Aust. J. Plant Physiol. **20**: 33-43, 1993.

Gucci, R., Massai, R., Xiloyannis, C., Flore, J.A.: The effect of drought and vapour pressure deficit on gas exchange of young kiwifruit (*Actinidia deliciosa* var. *deliciosa*) vines. – Ann. Bot. **77**: 605-613, 1996.

Judd, M.J., McAneney, K.J., Trought, M.C.T.: Water use by sheltered kiwifruit under advective conditions. – New Zeal. J. agr. Res. **29**: 83-92, 1986a.

Judd, M.J., McAneney, K.J., Wilson, K.S.: Influence of water stress on kiwifruit growth. – Irrig. Sci. **10**: 303-311, 1986b.

Katerrji, N., Mastrorilli, M., Rana, G.: Analysis and improvement of water use efficiency for crops cultivated in the Mediterranean regions: the state of the art. – Eur. Comm. Sixth Framework Progr. Contract FP6-517620, 2006.

Krause, G.H., Weis, E.: Chlorophyll fluorescence and photosynthesis: The basics. – Annu. Rev. Plant Physiol. Plant mol. Biol. **42**: 313-349, 1991.

Maxwell, K., Johnson, G.N.: Chlorophyll fluorescence – a practical guide. – J. exp. Bot. **51**: 659-668, 2000.

Medina, C.L., Souza, R.P., Machado, E.C., Ribeiro, R.V., Silva, J.A.B.: Photosynthetic response of citrus grown under reflective aluminized polypropylene shading nets. – Sci. Hortic. **96**: 115-125, 2002.

Miller, S.A., Smith, G.S., Boldingh, H.L., Johansson, A.: Effects of water stress on fruit quality attributes of kiwifruit. – Ann. Bot. **81**: 73-81, 1998.

Moran, R.: Formulae for determination of chlorophyllous pigments extracted with *N,N*-dimethylformamide. – Plant Physiol. **69**: 1376-1381, 1982.

Powles, S.B.: Photoinhibition of photosynthesis induced by visible light. – Annu. Rev. Plant Physiol. **35**: 15-44, 1984.

Quero, J.L., Villar, R., Marañón, T., Zamora, R.: Interactions of drought and shade effects on seedlings of four *Quercus* species: physiological and structural leaf responses. – New Phytol. **170**: 819-834, 2006.

Šircely, H., Tauszb, M., Grill, D., Batič, F.: Biochemical responses in leaves of two apple tree cultivars subjected to progressing drought. – J. Plant Physiol. **162**: 1308-1318, 2005.

Terzaghi, W.B., Fork, D.C., Berry, J.A., Field, C.B.: Low and high temperature limits to PS II. A survey using *trans*-parinaric acid delayed light emission, and $F_0$ chlorophyll fluorescence. – Plant Physiol. **91**: 1494-1500, 1990.

Turner, N.C.: Techniques and experimental approaches for the measurement of plant water status. – Plant Soil **58**: 339-366, 1981.

Valladares, F., Pearcy, R.W.: Interactions between water stress, sun-shade acclimation, heat tolerance and photoinhbition in the sclerophyll *Heteromeles arbutifolia.* – Plant Cell Environ. **20**: 25-36, 1997.

Werner, C., Ryel, R.J., Correia, O., Beyschlag, W.: Structural and functional variability within the canopy and its relevance for carbon gain and stress avoidance. – Acta oecol. **22**: 129-138, 1999.