

Interactions between water stress, sun-shade acclimation, heat tolerance and photoinhibition in the sclerophyll *Heteromeles arbutifolia*

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ABSTRACT

Gas exchange and chlorophyll fluorescence techniques were used to evaluate the acclimation capacity of the sclerophyll shrub *Heteromeles arbutifolia* M. Roem. to the multiple co-occurring summer stresses of the California chaparral. We examined the influence of water, heat and high light stresses on the carbon gain and survival of sun and shade seedlings via a factorial experiment involving a slow drying cycle applied to plants grown outdoors during the summer. The photochemical efficiency of PSII exhibited a diurnal, transient decrease ($\Delta F/F_m'$) and a chronic decrease or photoinhibition (F_v/F_m) in plants exposed to full sunlight. Water stress enhanced both transient decreases of $\Delta F/F_m'$ and photoinhibition. Effects of decreased $\Delta F/F_m'$ and F_v/F_m on carbon gain were observed only in well-watered plants since in water-stressed plants they were overridden by stomatal closure. Reductions in photochemical efficiency and stomatal conductance were observed in all plants exposed to full sunlight, even in those that were well-watered. This suggested that *H. arbutifolia* sacrificed carbon gain for water conservation and photoprotection (both structurally via shoot architecture and physiologically via down-regulation) and that this response was triggered by a hot and dry atmosphere together with high PFD, before severe water, heat or high PFD stresses occur. We found fast adaptive adjustments of the thermal stability of PSII (diurnal changes) and a superimposed long-term acclimation (days to weeks) to high leaf temperatures. Water stress enhanced resistance of PSII to high temperatures both in the dark and over a wide range of PFD. Low PFD protected photochemical activity against inactivation by heat while high PFD exacerbated damage of PSII by heat. The greater interception of radiation by horizontally restrained leaves relative to the steep leaves of sun-acclimated plants caused photoinhibition and increased leaf temperature. When transpirational cooling was decreased by water stress, leaf temperature surpassed the limits of chloroplast thermostability. The remarkable acclimation of water-stressed

plants to high leaf temperatures proved insufficient for the semi-natural environmental conditions of the experiment. Summer stresses characteristic of Mediterranean-type climates (high leaf temperatures in particular) are a potential limiting factor for seedling survival in *H. arbutifolia*, especially for shade seedlings lacking the crucial structural photoprotection provided by steep leaf angles.

Key-words: *Heteromeles arbutifolia*; heat stress; interaction between stresses; leaf angle; photoinhibition; photosynthesis; sclerophyll; sun-shade acclimation; thermal stability of photosystem II; transpiration cooling; water stress.

Abbreviations: *A*, net CO₂ assimilation rate; F_v/F_m , photochemical efficiency of PSII (dark adapted leaves); $\Delta F/F_m'$, photochemical efficiency of PSII in the light; g_w , stomatal conductance to water vapour; PFD, photosynthetic photon flux density; PSII, photosystem II; T_c , critical temperature for heat-induced fluorescence rise; T_p , temperature of heat-induced peak fluorescence; Ψ , water potential.

INTRODUCTION

In Mediterranean-type climates, plants are typically subjected to water and heat stress during the summer (Nahal 1981). The scarcity of precipitation during this season is generally associated with a high frequency of clear days, which translates into large solar radiation loads. Leaf overheating is invariably the result of a large influx of absorbable energy combined with insufficient loss of heat, solar radiation being the most important energy source under natural conditions (Larcher 1995). In addition, strong light can photoinhibit photosynthesis and may lead to photooxidative destruction of the photosynthetic apparatus (Powles 1984; Osmond 1994). Although it may be convenient for the physiologist to consider the plant response to various stresses separately, most responses are neither independent nor specific. The facts that multiple stresses co-occur and that the response to several simultaneous stresses is usually not predictable by single-factor analyses make the study of the interactions both appropriate and complex (Björkman 1987; Gamon & Pearcy 1990b). A combination of different stress factors can result in intensification, overlapping or reversal of the stress effects

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(Osmond *et al.* 1986). Leaf photosynthesis is easily abolished by elevated temperatures (Berry & Björkman 1980), but high temperature and high PFD have a more profound effect on PSII photochemical activity together than they have separately (Ludlow 1987). The relationships between water stress and other environmental factors can be very intricate. For instance, as long as sufficient water is available, leaf overheating can be prevented by transpirational cooling (Larcher 1995). Since transpiration is drastically reduced by drought, it has proved very difficult to separate the direct effects of high temperature from those of water deficit (Gates 1968). To complicate the matter further, leaf desiccation enhanced resistance of PSII to high temperature stress in certain species (Havaux 1992). Stomatal closure caused by water stress inhibits photosynthesis by limiting the availability of CO₂ within the leaf (Boyer 1976; Chaves 1991). In addition, water stress may predispose leaves to photoinhibition (Björkman & Powles 1984; Ludlow & Björkman 1984) since photosynthesis can be impaired by non-stomatal effects under low leaf water potentials (mainly reductions in PSII activity and electron transfer reactions; see reviews of Cornic 1994; Ort *et al.* 1994). However, the nature of these biochemical limitations related to water deficits, and the question of whether they really occur extensively *in vivo*, are controversial because these limitations have not been found in all studies (Sharp & Boyer 1986; Gamon & Pearcy 1990b) and could be artefacts or just the result of photoinhibition occurring during the water limitation (Cornic 1994).

Prevention of dangerous overheating and photoinhibition can be achieved by certain morphological mechanisms such as steep leaf angles (Mooney *et al.* 1977; Comstock & Mahall 1985; Lovelock & Clough 1992). A field study indicated that the main structural difference between sun and shade populations of the chaparral sclerophyll *Heteromeles arbutifolia* M. Roem. was the leaf angle, which in sun-acclimated plants was very steep and provided remarkable photoprotection by reducing the interception of radiation that could not be used in photosynthesis (Valladares & Pearcy, unpublished results).

The main objective of this study was to ascertain the acclimation capacity of *Heteromeles arbutifolia* to the multiple co-occurring summer stresses of the California chaparral and to assess their influence on the carbon gain and survival of seedlings. Using a factorial experimental design, we were able to explore both single factor effects and interactions between stresses. By manipulating leaf angles, we eliminated the structural photoprotection provided by steep leaf angles in order to elucidate the role of structural stress prevention versus physiological stress tolerance. We included the acclimation to sun or shade as another factor in the experimental design. Chaparral shrubs such as *H. arbutifolia* are typically sun-acclimated plants when adults, but frequently establish in the shade of other plants; seedlings should therefore be especially vulnerable to reductions in carbon gain associated with chronic photoinhibition due to their lack of stored resources (Ball *et al.* 1991). Boyer *et al.* (1987) suggested that photoinhibition

may not be a large contributor to photosynthetic losses in water-limited plants because stomatal closure overrides the possible reductions of PSII activity under high PFD and water stress. However, Mooney (1989) considered that photoinhibition during the drought period has not been explored adequately in relation to evergreen sclerophylls.

MATERIALS AND METHODS

Plant material and experimental design

A factorial experiment of three factors of two levels each (eight experimental units or combinations) was designed to test for main effects and interactions on several physiological variables measured throughout a slow drying cycle. The factors and levels were: water stress versus well-watered treatment, light acclimation (sun and shade-acclimated plants) and light exposure (high and low PFD, i.e. sun and shade sites). Four plants were used for each combination and the average of two leaves was used as the value for each plant. Leaves were restrained horizontally with a thin, flexible wire, which prevented shading by neighbouring leaves and ensured that all leaves studied received the same PFD at the same time of the day (plant architecture and leaf angle differed dramatically between sun- and shade-acclimated plants, affecting patterns of PFD interception at the leaf level). A complete set of measurements before manipulating the leaf angle and introducing water restrictions was carried out as a reference (day 0).

One-year-old, 20-cm-high seedlings of *Heteromeles arbutifolia* M. Roem. were obtained from a local nursery and transplanted to large pots (22 dm³) in early January 1994. The pots were placed outdoors, in the facilities of the Greenhouse Area of the University of California at Davis, under natural PFD, temperature and humidity conditions. The plants were grown in a 50/50 (v/v) mixture of pumice rock and University of California mix potting soil with slow-release nutrient pellets (Osmocote). Prior to the initiation of the drying cycle the plants were kept well-watered. A metal frame with several layers of neutral shade cloth was placed over half of the plants to produce a low-light environment ('shade'). The design of the frame and the number of layers of shade cloth were optimized to avoid any effect on the temperature of the air in contact with the plants and to obtain the same background PFD experienced by natural shade populations of *H. arbutifolia* found in the nearest chaparral formation (G.L. Stebbins Cold Canyon Reserve of the University of California Natural Land and Water Reserve System). The average photosynthetic photon flux density (PFD) under the shade cloth during the central hours of the day was 10–15% full sunlight (Fig. 1). Plants were 1.5–1.7 m tall after 6 months of growth and 90–95% of the leaves present at that time were formed during this period. Water stress and systematic measurements of various physiological parameters were conducted during July 1994. July was chosen to explore the interactions between water, temperature and light stresses because it is the hottest and driest month of the year in the central valley

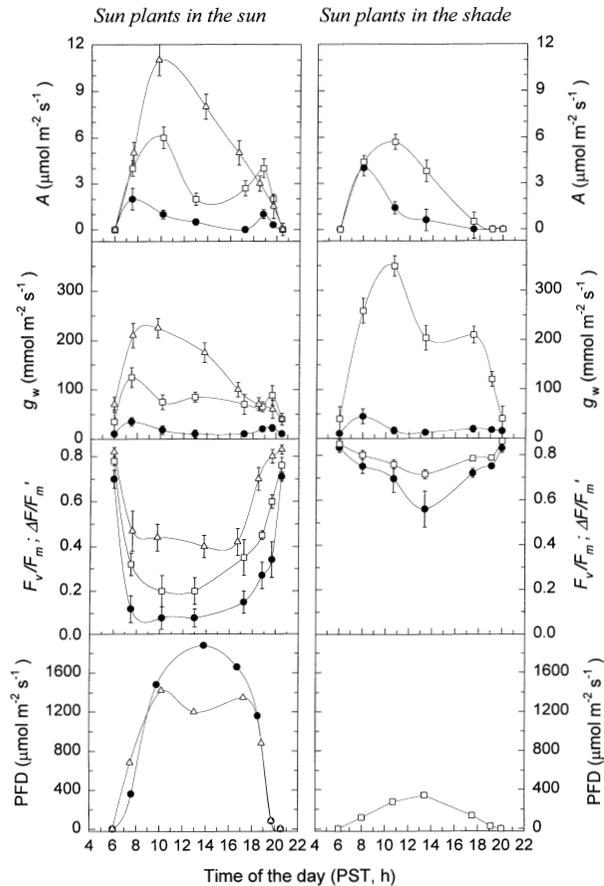


Figure 1. Diurnal course of net CO₂ assimilation (A), stomatal conductance (g_w), photochemical efficiency of PSII in the dark (F_v/F_m , pre-dawn and after-sunset measurements) and in the light ($\Delta F/F_m'$, the rest of the measurements), and photosynthetic photon flux density (PPFD) intercepted by single leaves of sun-acclimated plants during day 0 (before restraining the leaves horizontally and starting the induction of the water stress; open triangles) and day 19 (day of lowest leaf water potential in the water-stressed plants). Values for plants in the sun and in the shade and well-watered (open squares) and water-stressed (solid circles) are plotted independently. Bars give ± 1 SE.

of California. Solar radiation during July 1994 (Davis weather station) typically exceeded $502 \text{ kW m}^{-2} \text{ d}^{-1}$, with a diurnal average relative humidity below 30% and a maximal air temperature above $40 \text{ }^\circ\text{C}$. No cloudy days were observed to occur during this month. Daily PPFD during the experiments in July was on average $52.7 \text{ mol m}^{-2} \text{ d}^{-1}$ in the exposed site ('sun') and $8.1 \text{ mol m}^{-2} \text{ d}^{-1}$ in the shade.

Water stress induction and recovery

Half of the plants (four experimental units, i.e. 16 individuals) were submitted to a slow drying cycle, while the other half were watered to field capacity twice a day. The water stress was gradually increased until the values of midsummer leaf water potential reported for *H. arbutifolia* in the nearby Cold Canyon chaparral formation (Calkin & Pearcy 1984a; Calkin & Pearcy 1984b) were reached. The drought

cycle developed over 19 d, which should be slow enough to allow for osmotic and other physiological adjustments to drought. After induction of water stress (day 1), the pots with the plants were weighed every night and an amount equivalent to half of the evapo-transpired water was added. The complete lack of precipitation during the measurement period allowed for optimum control of the water regime of the plants. The water stress of the plants was assessed by periodic measurements of midday stem water potential and pre-dawn leaf water potential with a Scholander pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, California, USA). Water loss from the excised leaves was limited by wrapping them in a small plastic bag, and by humidifying the pressure chamber with a wet paper towel (Turner & Long 1980). Stem water potential was determined by measuring the water potential of leaves located near a main branch, which had been enclosed before dawn in black plastic bags covered with aluminium foil (Begg & Turner 1970). Ten leaves from each experimental unit were measured for each parameter. Stem water potential has been shown to be a sensitive and reliable plant-based measure of water stress in woody plants, and is less variable than leaf water potential (McCutchan & Shackel 1992). Irrigation of water-stressed plants was re-initiated once they reached the level of water stress observed in natural populations (day 19). The recovery was followed until leaf and stem water potentials stabilized around the values obtained on the first day of measurements. The recovery took 13 d, so the complete experiment lasted 32 d.

Diurnal gas exchange, chlorophyll fluorescence, light and leaf temperature measurements

Natural daily courses of gas exchange and chlorophyll fluorescence measurements were conducted through the drying and recovery cycles on two leaves per plant. Measurements of net photosynthesis *in situ* were carried out with a portable infrared gas analyser (LI-6200, LI-COR, Inc., Lincoln, Nebraska, USA). Single, attached, mature leaves were kept inside the cuvette under ambient radiation until three photosynthetic measurements had been recorded. The measurement process typically took less than 1 min, and no significant increase in the temperature within the cuvette was observed during this brief period. The mean of the three readings was used for comparisons and statistics. Stomatal conductance was measured with a portable steady state porometer (LI-COR model -1600). Concurrent measurements of chlorophyll fluorescence were made using a portable, pulse-modulated fluorometer (PAM 2000, Walz, Effeltrich, Germany). The fluorometer was operated via a notebook computer (Zeos Inc. USA). The measuring modulated red light was low enough ($c. 0.1 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$) to prevent any induction of variable fluorescence. The internal halogen lamp of the fluorometer was used at an intensity above $4000 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ for the short (800 ms) pulses of saturating light. Minimal (F_o) and maximal (F_m) fluorescence were measured at pre-dawn and 1 h after sunset from the upper side

of each leaf, and the photochemical efficiency of photosystem II (PSII) in darkness (F_v/F_m) was calculated. Steady-state fluorescence (F) and maximal fluorescence in the light (F_m') were sampled at various times during the day and the PSII quantum yield in the light [$(F_m' - F)/F_m' = \Delta F/F_m'$] was calculated according to Genty *et al.* (1989). Fluorescence nomenclature follows Van Kooten & Snel (1990). The fluorescence measurements of dark-adapted leaves were made using homemade clip holders with a black cylinder slightly wider than the fibre-optics. This holder blocked ambient light from the leaf and held the fibre-optics in the correct position during the measurement. For measurements of fluorescence under ambient light, we used a leaf clip holder (Walz) that held the fibre-optics at an angle of 60° and allowed adjustment of the fibre-optic distance for optimal signal without shading of the measuring zone (Bilger *et al.* 1995).

Leaf temperature was measured with 0.07-mm-diameter copper-constantan thermocouples attached to the lower side of the leaves and connected to a datalogger (Model CR21X, Campbell Scientific, Logan, UT, USA). Small GaAsP PFD sensors (Hanamatsu model G1118) attached to the upper side of the leaves were used to measure incident PFD. These sensors were calibrated against a quantum sensor (model LI-190SA, LI-COR) and were also connected to the datalogger. Temperature and PFD were measured on all plants and the readings were recorded every minute throughout the drying and recovery cycles.

Measurements of the fluorescence response of leaf discs to temperature and light

It was reported that a slow elevation of leaf temperature brings about a progressive increase in the chlorophyll *a* fluorescence intensity under low excitation light, reaching a peak at a temperature T_p presumably corresponding to a complete destruction of PSII activity (Schreiber & Berry 1977). Later studies demonstrated that the critical temperature for heat-induced fluorescence (T_c) and T_p are direct indices of the chloroplast thermostability (Berry & Björkman 1980) and can be used to estimate the relative heat tolerance of plants (Bilger *et al.* 1984). Leaves of the various study plants of *H. arbutifolia* were placed on wet filter paper in a Petri dish and kept in the dark, at room temperature (25 °C) for at least 1 h before fluorescence measurements were initiated. Discs of 2 cm² were excised from the central part of the leaf blades and placed on a thermostatted aluminium block. A home-made temperature controller with a temperature ramp function provided power to the heater and ramped the temperature up at 1 °C min⁻¹. A piece of moist filter paper was placed between the leaf disc and the aluminium block, and a glass cover was placed over the leaf disc. F_o in the presence of far-red light was recorded throughout the temperature rise experiment by a PAM 2000 fluorimeter and plotted on a strip chart recorder. Leaf temperature was monitored continuously by a digital thermocouple thermometer, with the thermocouple inserted in between the filter paper and the leaf

disc. T_c was calculated from the F_o versus temperature plots as in Bilger *et al.* (1984). T_c experiments were carried out on days 11–14 for well-watered plants, and days 16–18 (when water potentials were close to their lowest values) for water-stressed plants. T_c measurements were conducted again 1 month later, when the plants had apparently recovered from the water stress (leaf water potentials were the same as in well-watered plants) and were experiencing lower ambient temperatures (see below).

Since heat stress in nature usually takes place under medium to high PFD, we wished to explore its interactions with the PFD level. A different protocol was designed for this objective and changes in F_v/F_m as a result of exposure to different combinations of temperature (30–54 °C) and PFD (0–1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$) for various durations (1–60 min) were measured. Leaf discs were submitted to each temperature-PFD treatment in a small cuvette whose temperature was controlled by a continuous flow of water from a thermostatted water bath. A 1000 W metal halide lamp was used as the PFD source with the irradiance controlled by wire-screen, neutral-density filters. Room air was constantly pumped into the cuvette to maintain CO₂ concentrations. The temperature of the leaf discs was monitored with a thermocouple attached to the lower side of the leaf disc. Oscillations of disc temperature around the desired value were lower than 0.2 °C. After the temperature-light treatment, the leaf discs were allowed to recover under low PFD (65 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and room temperature (25 °C) in a Petri dish for 1.5 h. The discs were then kept in the dark for an additional hour before F_v/F_m was measured. Leaf discs were placed on a filter paper that was kept constantly wet both in the cuvette and in the Petri dishes. Control leaf discs not submitted to the temperature-PFD treatment but to all remaining steps of the protocol always exhibited F_v/F_m values around or slightly above 0.8, which we take as an indication of a reasonable lack of artifacts due to the manipulations themselves. This last series of experiments was conducted during late August and early September 1994. All plants studied were kept well-watered after the end of the drying cycle (late July). Air temperature was lower (typically around 30 °C) and daylength shorter at this time of the year, but the days were still sunny and dry (no significant cloudiness or precipitation took place until late September 1994).

Statistics

The effects of the various factors considered (water regime, light acclimation, PFD treatment and temperature) on the physiological parameters studied and the significance of the interactions were analysed by means of multi-factorial ANOVA (Sokal & Rohlf 1995). SigmaStat (Jandel Scientific, San Rafael, CA, USA) was used for most of the calculations. Statgraphics (STSC Inc. Rockville, MD, USA) was used for ANOVA of more than two factors. Data sets were tested for normality and equal variance (Kolmogorov-Smirnov and Cochran's C tests, respectively) and a log transformation was applied when

significant discrepancies from normality were found. Multiple comparisons among groups of individuals were carried out by Student-Newman-Keuls tests of paired comparisons.

RESULTS

Effects of PFD and water regime on gas exchange, photochemical efficiency and leaf temperature

Leaves of sun-acclimated plants restrained in a horizontal position for 19 d exhibited a midday depression in net assimilation rate (A) while the diurnal course of A observed in steep leaves before the experiments (day 0) was dome-

shaped (Fig. 1). The reverse was true for the diurnal pattern of PFD interception by the leaves (Fig. 1). Maximum rates of A (A_{\max}) reached values of $11 \mu\text{mol m}^{-2} \text{s}^{-1}$ during day 0 and decreased to $6 \mu\text{mol m}^{-2} \text{s}^{-1}$ after 19 d in well-watered plants. The transient diurnal decreases of the quantum efficiency of PSII ($\Delta F/F_m$) in response to increasing irradiance were more pronounced after 19 d of horizontal restraint of the leaves (Fig. 1) and F_v/F_m at pre-dawn was slightly but significantly lower (Fig. 2). The stomatal conductance to water vapour (g_w) matched the changes observed in the time course of A . Daily carbon fixation of these leaves was reduced by *c.* 50% after 2 weeks of intercepting full sunlight at midday and maximal g_w ($g_{w,\max}$) was also 50% of $g_{w,\max}$ on day 0. Midday depression of A and decreases in daily carbon fixation, g_w , F_v/F_m and

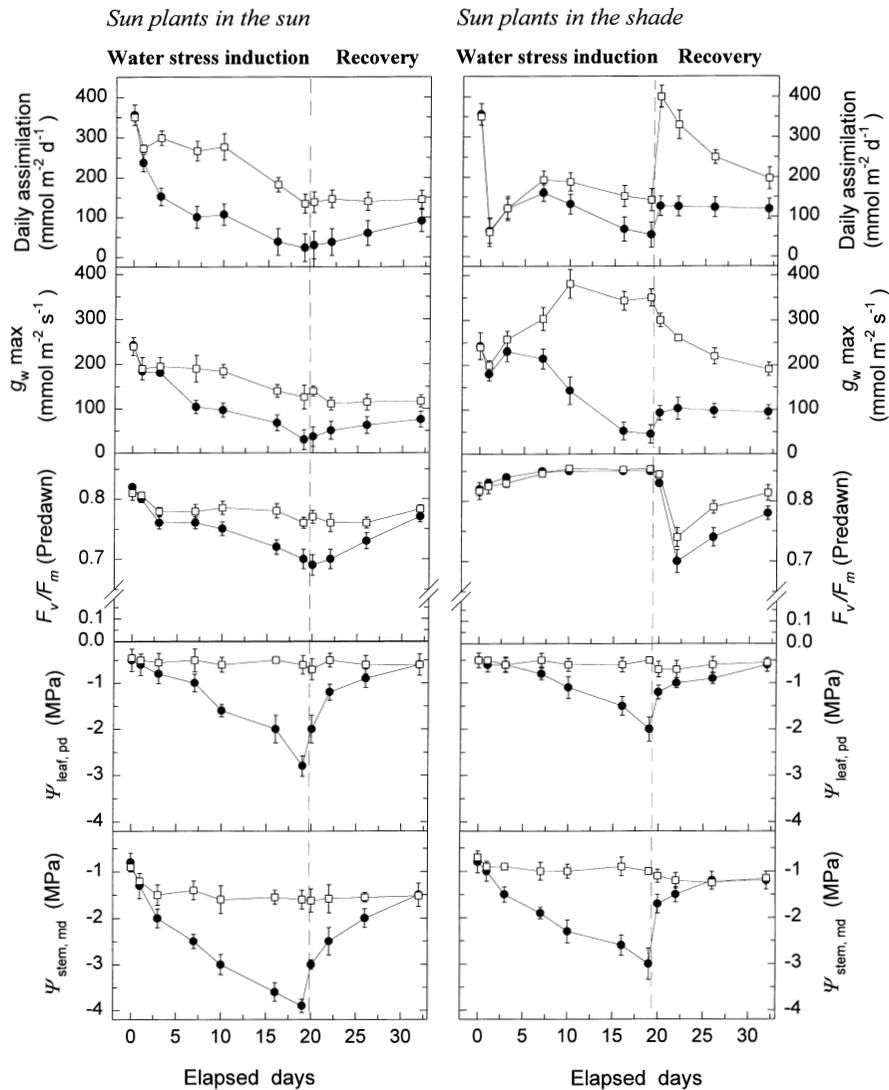


Figure 2. Time course of daily net CO₂ assimilation (A), maximum stomatal conductance ($g_{w,\max}$), photochemical efficiency of PSII in the dark (F_v/F_m at pre-dawn), pre-dawn leaf water potential ($\Psi_{\text{leaf, pd}}$) and midday stem water potential ($\Psi_{\text{stem, md}}$) of sun-acclimated plants in the sun and in the shade throughout a drying–recovery cycle. Values for well-watered (open squares) and water-stressed (solid circles) plants are plotted independently. Recovery of all sun-acclimated plants was conducted in the sun site. After day 0, leaves of all plants were restrained horizontally. Bars give ± 1 SE.

$\Delta F/F_m'$ were significantly larger in water-stressed plants than in well-watered plants (Figs 1 & 2). Daily carbon fixation and $g_{w,max}$ of leaves of water-stressed plants on day 19 were c. 10% of the values obtained on day 0. Measurements of control leaves of well-watered sun-acclimated plants that remained at their normal steep orientation revealed no significant decrease in A_{max} or $g_{w,max}$ over the period from 0 to 19 d. Thus the observed decrease for the well-watered plants over this period was a result of the reorientation and presumably the extra irradiance they received.

Sun-acclimated plants transferred to the shade exhibited a general increase in $g_{w,max}$, F_v/F_m and $\Delta F/F_m'$ but the daily carbon fixation was reduced as a result of PFD limitations relative to day 0 (Figs 1 & 2). This was true for both well-watered and water-stressed plants. Water stress reduced daily carbon gain, $g_{w,max}$ and $\Delta F/F_m'$ but did not affect the pre-dawn and after-sunset F_v/F_m of sun-acclimated plants in the shade.

The A_{max} and daily carbon gain of shade-acclimated plants in the shade were about half of the values for sun-acclimated plants in the sun, while g_w was approximately the same (Figs 3 & 4). Consequently, the water use efficiency of shade-acclimated plants was significantly lower than that of sun-acclimated plants in their respective light environments (data not shown). Pre-dawn values of F_v/F_m were slightly but significantly higher in shade-acclimated plants than in sun-acclimated plants in their respective light environments. When transferred to the sun, shade-acclimated plants exhibited a brief increase in daily carbon gain followed by a decrease associated with a chronic decrease in F_v/F_m (Fig. 4). However, after 5 d in the sun, both F_v/F_m and daily carbon gain recovered to some extent in leaves of well-watered plants. Water stress accentuated these changes and caused a remarkable reduction of stomatal conductance. No recovery of daily carbon gain and F_v/F_m was observed in water-stressed shade-acclimated plants until they were transferred back to the shade and the irrigation reinitiated.

By 13 d after reinitiating the irrigation (day 32, Fig. 2), the pre-dawn leaf water potential and midday stem water potential of all water-stressed plants recovered to values equal to those of plants kept well-watered. However, the daily carbon gain and stomatal conductance of these plants did not recover completely to the values of well-watered plants. Pre-dawn F_v/F_m of water-stressed plants recovered to the values of well-watered plants, except in the case of sun-acclimated plants kept in shade (Fig. 2). In this case, F_v/F_m initially decreased after transfer back to the sun but the values of the water-stressed plants recovered to 0.78 whereas the well-watered plants recovered to 0.81. The highest photochemical efficiency ($F_v/F_m = 0.84$) and $g_{w,max}$ were measured in well-watered sun-acclimated plants kept in the shade. After being kept for 19 d in the shade, but then returned to the sun, the well-watered sun-acclimated plants exhibited temporarily the largest daily carbon gain of all plants studied (Fig. 2).

The average leaf temperature (from 1130 to 1630 h) of

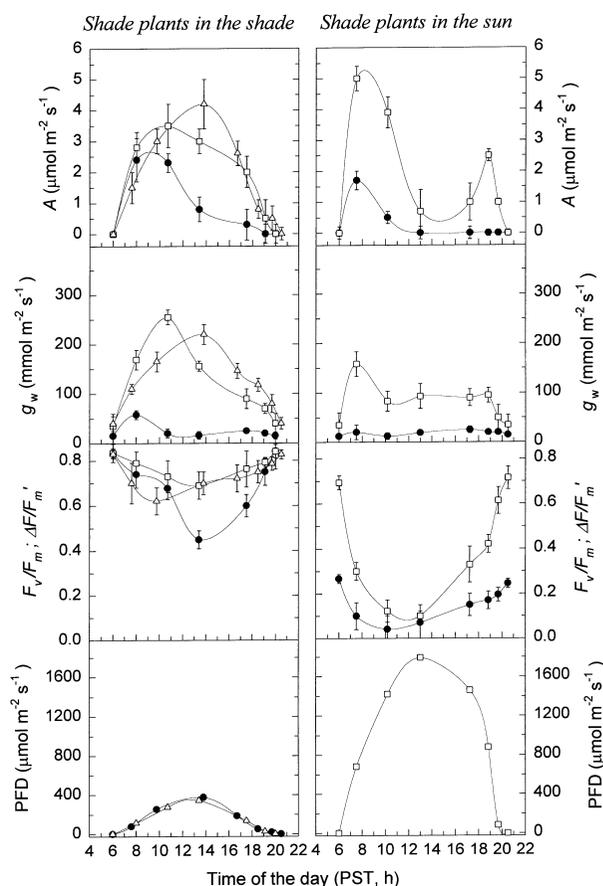


Figure 3. Diurnal course of net CO₂ assimilation (A ; note the difference in scale with Fig. 1), stomatal conductance (g_w), photochemical efficiency of PSII in the dark (F_v/F_m , pre-dawn and after-sunset measurements) and in the light ($\Delta F/F_m'$, the rest of the measurements), and photosynthetic photon flux density (PFD) intercepted by single leaves of shade-acclimated plants during day 0 (before restraining the leaves horizontally and starting the induction of the water stress; open triangles) and day 19 (day of lowest leaf water potential in water-stressed plants). Values for plants in the shade and in the sun and well-watered (open squares) and water-stressed (solid circles) are plotted independently. Bars give ± 1 SE.

well-watered sun-acclimated plants was lower than the average air temperature at the beginning of the measurements due to transpirational leaf cooling (Fig. 5). Shade-acclimated plants transferred to the sun always exhibited leaf temperatures equal to or above air temperature. This was because they had a lower g_w and higher radiation interception, because of their lower leaf angles, than sun-acclimated plants. These initial differences between sun- and shade-acclimated plants disappeared after the sampled leaves had been held horizontal for 10 d. The leaf temperature in the shade was always close to air temperature. The reduction in g_w observed in water-stressed plants caused a significant increase in leaf temperature (Fig. 5). The maximal leaf temperature of water-stressed plants in the sun was up to 10 °C above air temperature and exceeded 50 °C on the hottest days.

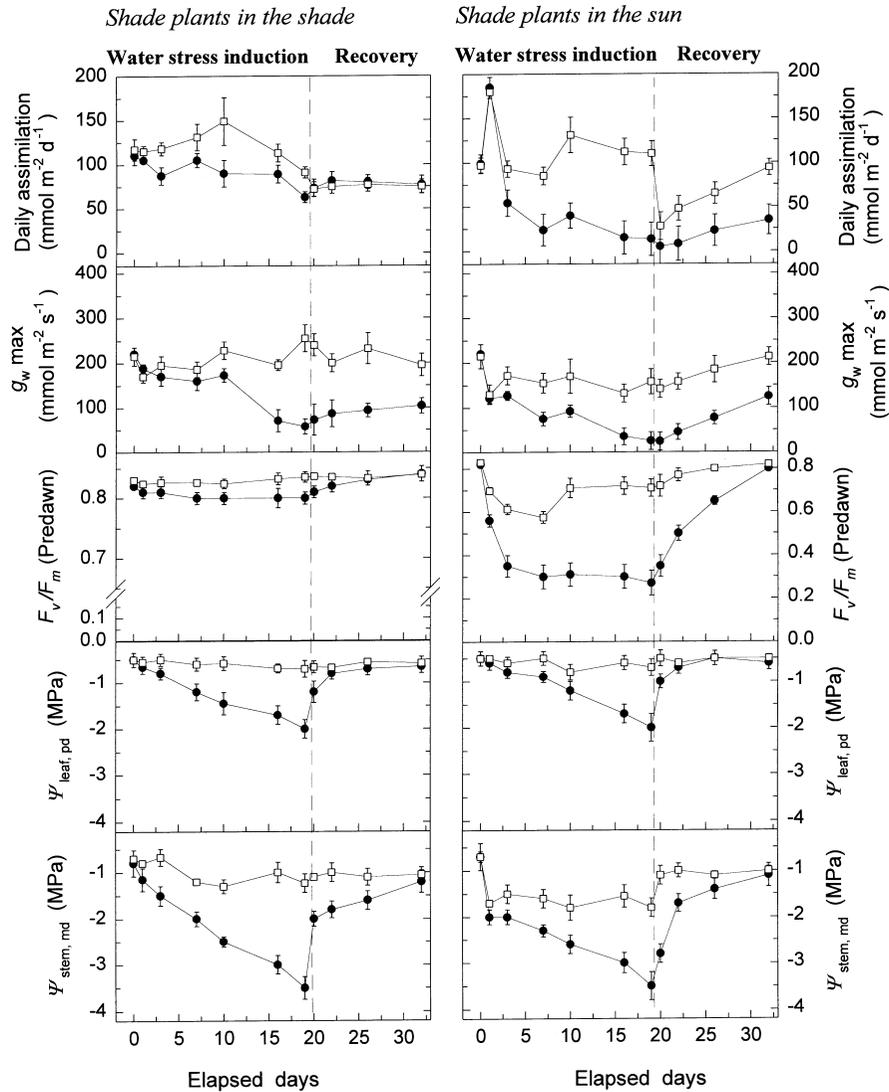


Figure 4. Evolution of net CO₂ assimilation (A; note the difference in scale from Fig. 2), maximal stomatal conductance (g_w max), photochemical efficiency of PSII in the dark (F_v/F_m at pre-dawn), pre-dawn leaf water potential ($\Psi_{leaf, pd}$) and midday stem water potential ($\Psi_{stem, md}$) of shade-acclimated plants in the shade and in the sun throughout a drying–recovery cycle. Values for well-watered (open squares) and water-stressed (solid circles) plants are plotted independently. Recovery of all shade-acclimated plants took place in the shade site. After day 0, leaves of all plants were restrained horizontally. Bars give ± 1 SE.

Thermal tolerance, PFD and water regime

The critical temperature for PSII (critical temperature for heat-induced fluorescence rise, T_c) exhibited diurnal changes coupled to changes in air temperature (Fig. 6). T_c was below 50 °C in the early morning and above 50 °C in the afternoon. T_c was generally higher in sun- than in shade-acclimated plants when well-watered individuals in their respective light environments were compared. Water-stressed plants always exhibited higher T_c than their well-watered counterparts, and this effect was maintained for more than 1 month after the reinitiation of the irrigation (Fig. 6). It is noteworthy that the maximal leaf temperature reached T_c on occasion for water-stressed plants kept in the sun.

High PFD had a synergistic effect on the reduction of the photochemical efficiency of PSII (F_v/F_m) by high temperatures in both shade- and sun-acclimated plants. High temperatures reduced F_v/F_m to a lower value when applied under high PFD (1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$) than when applied under low PFD (65 $\mu\text{mol m}^{-2} \text{s}^{-1}$) in all plants (Fig. 7). F_v/F_m was less affected by high PFD and temperatures between 30 and 48 °C in sun-acclimated plants than in shade-acclimated plants. Temperatures above 50 °C caused a dramatic decrease of F_v/F_m in plants from all treatments. Water-stressed plants exhibited higher values of F_v/F_m than well-watered plants when both PFD treatments were applied at temperatures of 43–48 °C (Figs 7 & 8). In particular, water-stressed sun-acclimated plants

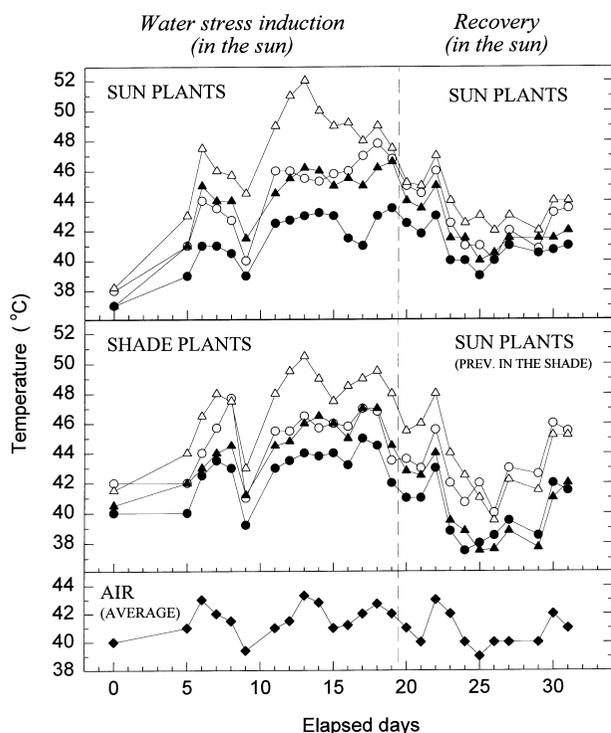


Figure 5. Time course of leaf temperatures of sun- and shade-acclimated plants in the sun throughout the drying and recovery cycle which were kept either well-watered (circles) or water-stressed (triangles). Temperatures are the mean (solid symbols) and maximal (open symbols) values registered during midday to early afternoon (1130–1630 h local time). Each point is the mean of measurements on four plants (one leaf per plant).

showed a remarkable tolerance to different combinations of PFD and temperature in comparison with the other groups of plants. All plants studied recovered to higher values of F_v/F_m when high temperature was applied under low PFD than when it was applied in the dark or under high PFD (Fig. 8). At least 70% of the observed reduction in F_v/F_m caused by high PFD plus high temperature took place in the first 10 min (Fig. 9).

A four-way factorial ANOVA of F_v/F_m after 1 h of treatment and 2.5 h of recovery (1.5 h under low PFD and 1 h in the dark) was carried out to test and confirm the interactions between the different factors studied. The four factors were: (i) PFD treatment (high = $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$, low = $65 \mu\text{mol m}^{-2} \text{s}^{-1}$), (ii) temperature treatment (high = 45°C , very high = 51°C), (iii) water regime (well-watered and water-stressed plants), and (iv) light acclimation (sun- and shade-acclimated plants). The results for single factors confirmed that each one independently had a significant effect on F_v/F_m (Table 1). As expected, the effect of PFD on F_v/F_m depended on the light regime to which each plant was acclimated (revealed by significant interactions between these two factors). The effect of water stress on F_v/F_m depended neither on the light treatment nor on the light acclimation. However, it did depend on temperature, since the F_v/F_m values of water-stressed and

well-watered plants after exposure to a temperature of 51°C were not significantly different (Table 1). The effect of temperature on F_v/F_m depended on the light regime to which the plant was acclimated, and on the PFD experienced.

DISCUSSION

The photochemical efficiency of PSII exhibited two different time responses to high radiation and water stresses: a diurnal, transient change (assessed by $\Delta F/F_m'$) and a chronic decrease (assessed by F_v/F_m) that we will refer to as chronic photoinhibition. The interpretation of a sustained decrease in the efficiency of PSII is difficult because a decline in F_v/F_m can be due to an increase in protective non-radiative energy dissipation (non-photochemical quenching), to photodamage of the PSII reaction centre complex, or to a combination of both (Osmond 1994). Non-photochemical quenching usually vanishes when light intensity decreases (Demmig-Adams & Adams 1992) but it can be either slowly reversible or irreversible under stress conditions (Ruban & Horton 1995). The origin of a sustained non-photochemical quenching has not been clearly established. Two different mechanisms have been proposed to explain this chronic quenching: changes in the structure and function of the light-harvesting chlorophyll proteins regulated by protonation and the ratio of zeaxanthin to violaxanthin (Ruban & Horton 1995), and a sustained de-epoxidation state of the xanthophyll cycle dependent on lumen acidification and chloroplastic ATP hydrolysis (Gilmore & Björkman 1995).

The strong transient decrease in $\Delta F/F_m'$ and its recovery in the evening observed in the present study were related to changes in irradiance (they were minor in the shade), and resulted from the combination of a reduced photochemical quenching and a decreased efficiency of open PSII units. Water stress enhanced this transient decrease of photo-

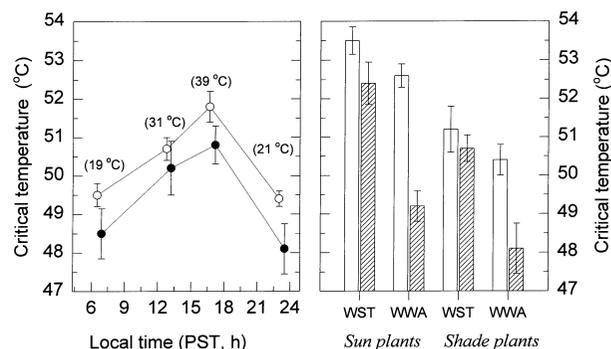


Figure 6. Left graph: diurnal evolution of the critical temperature for PSII (T_c) of sun-acclimated (open circles) and shade-acclimated (solid circles) plants in their respective light environments (air temperature in brackets). Right graph: T_c of well-watered (WWA) and water-stressed (WST) sun- and shade-acclimated plants on 16 July (average air temperature during the central hours of the day was 41°C ; open bars) and 4 September (average air temperature during the middle hours of the day was 30°C ; hatched bars). Each point is the average of four plants. Bars give ± 1 SD.

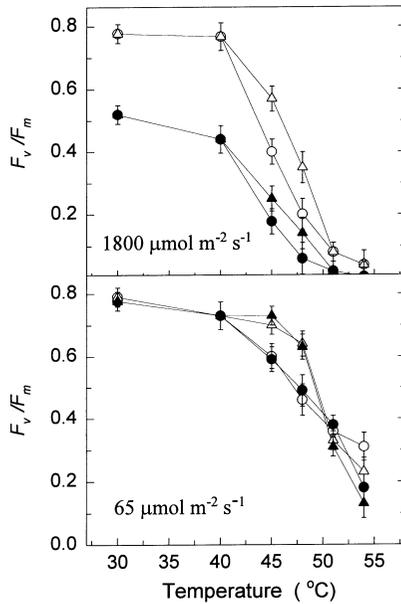


Figure 7. F_v/F_m after 1 h of PFD-temperature treatment and 2.5 h of recovery at room temperature (1.5 h under low PFD and 1 h in the dark) as a function of the temperature during the treatment. Results for two PFD (high PFD, upper graph, and low PFD, lower graph), for sun (open symbols) and shade (solid symbols) and for well-watered (circles) and water-stressed (triangles) plants are shown independently. Each point is the average of four plants. Bars give ± 1 SD.

chemical efficiency in *H. arbutifolia*, as has been observed in other wild plants that experience drought in their natural environments (Valentini *et al.* 1995). Transient decreases in $\Delta F/F_m'$ are a general feature of photosynthesis in natural environments (Demmig-Adams & Adams 1992) and are considered to be a down-regulation that maintains a balance between light-driven electron flow and requirements for reducing power required to support carboxylation and oxygenation (Krause & Weis 1991). Photoinhibition was observed in all horizontal leaves exposed to full sunlight and was also associated with a decrease in the carbon gain of well-watered plants of *H. arbutifolia*, especially of those previously acclimated to shade. Water stress further increased photoinhibition of all plants exposed to full sunlight. This synergistic effect of high PFD and water stress on photoinhibition agrees with the results obtained in *Nerium oleander* (Björkman & Powles 1984) and *Macroptilium atropurpureum* (Ludlow & Björkman 1984) but is in contrast to findings with *Vitis californica* (Gamon & Percy 1990b) and *Quercus cerris* (Valentini *et al.* 1995). It therefore appears that the effects of low water status on the susceptibility of PSII to photodamage are species-specific. However, the reasons why water stress disturbed the overnight recovery of the F_v/F_m of shade-acclimated plants of *H. arbutifolia* kept in the shade but not that of sun-acclimated plants kept in the shade are not understood. Photoinhibition and reduction of PSII activity by water deficit played little role in the carbon gain of water-stressed plants of *H. arbutifolia* since, as predicted

by Boyer *et al.* (1987), stomatal closure overrode the decrease in photochemical efficiency, impeding gas exchange far more than would have occurred due to reduction in PSII activity alone (Figs 2 & 4).

The main target of high temperature stress in vascular plants is photosynthetic functions. The heat tolerance limit of leaves coincides with the temperature at which thermal damage to the primary photochemical reactions of the thylakoid membrane system occurs (Weis & Berry 1988). We observed in *H. arbutifolia* fast adaptive adjustments of the thermal stability of PSII, occurring in the time range of a few hours (diurnal changes), which were superimposed on a long-term acclimation occurring over days to weeks (plants in the sun versus in the shade and water-stressed versus well-watered). Both types of adjustments have been observed before (Seeman *et al.* 1986; Weis & Berry 1988) and have been associated with protein phosphorylation and accumulation of heat shock proteins (rapid adjustments) and with changes in the composition of membrane lipids and changes in leaf osmotic potential (long-term adjustments). Havaux (1993) found a quick (0.5–1 h) enhancement of 5 °C in the thermal tolerance of PSII in potato leaves triggered by a slight change in leaf temperature (from 25 to 30 °C). The fast response of PSII thermoresistance to changes in leaf temperatures was less pronounced in *H. arbutifolia*: an increase of 20 °C (from 19 to 39 °C) in 10 h was associated with an increase in T_c of less than 3 °C (Fig. 6). However, T_c in potato leaves was much lower than the values of T_c obtained in *H. arbutifolia* (36.3–44.2 °C in comparison to 48.5–51.5 °C) so the requirements for large, transient adjustments of the thermal stability of PSII were very different in the two cases. Heat stress in natural conditions is usually progressive, allowing for a gradual heat-hardening. However, a relatively rapid adjustment of PSII thermotolerance to the prevailing temperatures is crucial for leaf survival in arid environments because transient

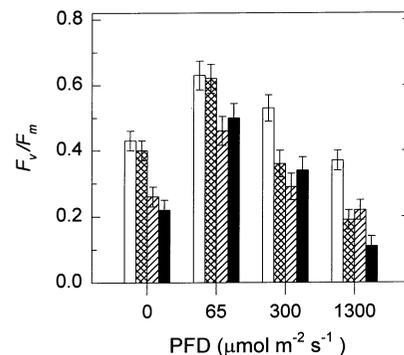


Figure 8. F_v/F_m after a light-temperature treatment and 2.5 h of recovery at room temperature (1.5 h under low PFD and 1 h in the dark) versus the light intensity during the treatment. Leaf discs were kept at 48 °C for 60 min. Open bars, sun-acclimated water-stressed plants; cross-hatched bars, shade-acclimated water-stressed plants; hatched bars, sun-acclimated well-watered plants; solid bars, shade-acclimated well-watered plants. Each point is the average of four plants. Bars give ± 1 SD.

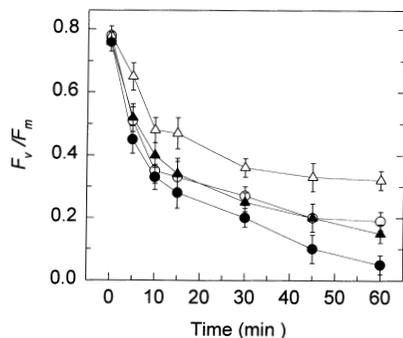


Figure 9. F_v/F_m after a light-temperature treatment and 2.5 h of recovery at room temperature (1.5 h under low PFD and 1 h in the dark) versus the duration of the treatment. Leaf discs were kept at 48 °C and $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$. Sun (open symbols) and shade (solid symbols) plants, and well-watered (circles) and water-stressed (triangles) plants are plotted independently. Each point is the average of four plants. Bars give ± 1 SD.

increases of leaf temperature can have dramatic effects: most of the damage observed in leaves of *H. arbutifolia* took place in less than 15 min (Fig. 9). In agreement with the findings of Havaux (1993), dynamic adaptation of the photosynthetic system to the rapidly changing temperatures of natural environments could be a more important component of the thermotolerance of plants than the intrinsic resistance of PSII.

Havaux (1992) demonstrated the existence of an antagonism between physicochemical stresses in detached, dehydrated leaves of *Lycopersicon esculentum* and *Solanum* sp. with water stress enhancing the resistance of PSII to high PFD at high temperature. Here we have shown a similar antagonism between water stress and high temperature in leaves of a wild sclerophyll shrub subjected to a water stress treatment that resembled natural conditions (slow and gradual water stress of whole plants, leaf water deficits, air humidity, temperature and radiation close to the values reported for nearby populations). Water stress enhanced the resistance of *H. arbutifolia* leaves to high temperature at any PFD, from darkness to $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$, and in both sun- and shade-acclimated plants, as indicated by the lack of interactions between water treatment and either light treatment or light acclimation (Table 1). As found in pea leaves (Havaux *et al.* 1991), light protected photochemical activity against inactivation by heat. This photoprotection was saturated at low PFD ($65 \mu\text{mol m}^{-2} \text{s}^{-1}$). High PFD ($> 1000 \mu\text{mol m}^{-2} \text{s}^{-1}$) had the opposite effect, enhancing the damage of PSII by heat (Fig. 8). These interactive effects of high temperatures and high PFD could well limit carbon gain in wild *H. arbutifolia* plants, as was suggested for the California wild grape (Gamon & Pearcy 1990a).

The greater interception of radiation during the middle hours of the day by horizontal leaves, relative to the normally steep leaves, caused photoinhibition and increased leaf temperature even in well-watered sun-acclimated plants (compare day 0, steep leaves, with subsequent

days, horizontal leaves, in Figs 2 & 5). When transpirational cooling was decreased by water stress, leaf temperature reached the limits of chloroplast thermostability. In fact, many horizontally restrained leaves of water-stressed plants exhibited necrotic spots that were putatively a consequence of overheating. These spots developed within 1 week after the beginning of the experiments. The remarkable acclimation of water-stressed plants to high leaf temperatures proved to be insufficient for the semi-natural environmental conditions of the experiment. Therefore, summer stresses characteristic of Mediterranean-type climates (high leaf temperatures in particular) are potential limiting factors for seedling survival in *H. arbutifolia*. This is especially so for seedlings acclimated to the shade that are exposed to full sunlight during the summer, since, in addition to their higher sensitivity to photoinhibition, their leaves are almost horizontal. The ecological implications of light in the chaparral community have been traditionally addressed in terms of competition for light and of the proportion of photosynthates that can be allocated to roots, which determines drought survival (e.g. Mahall & Schlesinger 1982), but very seldom in terms of the deleterious effects of excessive radiation on seedling survival. Shade features of leaves and whole shoots of *H. arbutifolia* increase light harvesting and utilization efficiencies (Valladares & Pearcy, unpublished results) but jeopardize survival under full sunlight. The shoot architecture of sun-acclimated plants of *H. arbutifolia*, with steep

Table 1. Four-factor ANOVA of F_v/F_m after 1 h of treatment and 2.5 h of recovery at room temperature (1.5 h under low PFD and 1 hour in the dark). The treatments were high PFD ($1800 \mu\text{mol m}^{-2} \text{s}^{-1}$) and low PFD ($65 \mu\text{mol m}^{-2} \text{s}^{-1}$), high temperature (45 °C) and very high temperature (51 °C), and were applied to samples of plants under two water regimes (well-watered and water-stressed) and two light regimes (full sunlight and shade). The experiments were conducted 1 month after the end of the drying-recovery cycle, and no significant differences in leaf water potentials were found between groups of plants at the time of measurements. Degrees of freedom = 1, $n = 4$ plants (average of 2 leaves per plant). Only results for main effects and first-order interactions are shown

Source of variation	F statistics	Significance
Light treatment (factor L)	910.3	$P \ll 0.01$
Temperature treatment (factor T)	999.5	$P \ll 0.01$
Water regime (factor W)	8.4	$P < 0.01$
Sun-shade (factor S)	110.9	$P \ll 0.01$
L \times T	6.2	$P < 0.05$
L \times W	0.4	not significant
L \times S	95.1	$P \ll 0.01$
T \times W	140.7	$P \ll 0.01$
T \times S	18.7	$P < 0.01$
W \times S	0.7	not significant

leaves, provided a structural photoprotection that proved essential in avoiding temperatures and PFD beyond the acclimation limits of the leaves, and this photoprotection was provided with few costs in terms of potential carbon gain (Valladares & Pearcy, unpublished results).

H. arbutifolia has roots of intermediate depth and experiences very high water stress during the summer in the chaparral communities (Calkin & Pearcy 1984; Davis & Mooney 1986). It can be defined as a drought tolerator, in contrast to some chaparral trees (e.g. *Quercus* spp.) that are basically drought avoiders (Davis & Mooney 1986). A high efficiency in the use of water is vital for its survival, as is the case for many co-occurring shrubs (Mahall & Schlesinger 1982). *H. arbutifolia* reduced transpiration by closing stomata in response to water stress, which in turn reduced both CO₂ exchange and transpirational cooling. However, even well-watered plants reduced transpiration in full sunlight, avoiding complete opening of the stomata: this is demonstrated by the fact that $g_{w,max}$ reached significantly larger values when the same well-watered individuals were transferred to the shade. Plants in the sun exhibited a general down-regulation of photosynthetic processes even in the absence of pronounced heat, high PFD or water stresses. Photochemical efficiency (assessed by F_v/F_m) and daily carbon gain were slightly but significantly down-regulated in sun-acclimated plants, even when the structural photoprotection provided by steep leaf angles was not prevented, as revealed by the increase of F_v/F_m when they were transferred to the shade and by the record carbon gain measured on the first day in the sun after transfer from the shade. A combination of low stomatal conductance and enhanced non-photochemical quenching (data not shown) accounted for these down-regulations. Thus, *H. arbutifolia* sacrificed carbon gain for water conservation and photoprotection (both structurally via shoot architecture and physiologically via energy dissipation) and this response is presumably triggered by a hot and dry atmosphere together with high PFD, before severe water, heat or high PFD stresses occur. This preventive strategy seems very adaptive in environments with unpredictability of limiting resources, for example rain in Mediterranean-type climates, and with multiple co-occurring stresses. The fact that some features of water-stressed plants (low stomatal conductance, high T_c) were maintained for weeks after normal leaf water potentials were recovered further emphasizes the importance of stress prevention versus yield maximization in seedlings of *H. arbutifolia*.

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