

Drought can be more critical in the shade than in the sun: a field study of carbon gain and photo-inhibition in a Californian shrub during a dry El Niño year

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ABSTRACT

Diurnal courses of leaf water potential (Ψ), gas exchange and chlorophyll fluorescence were measured in natural sun and shade populations of *Heteromeles arbutifolia* throughout the seasons of an unusually dry El Niño year in Central California. The onset of drought resulted in decreased stomatal conductance and net photosynthesis in both sun and shade plants. However, the decline in Ψ was much greater and carbon gain was much more strongly limited by the development of drought stress in the shade than in the sun. Photorespiratory energy dissipation was significantly higher in the sun than in the shade in spring and autumn, but not during the summer. Pre-dawn photochemical efficiency (F_v/F_m) was significantly higher in the shade than in the sun during the spring but the differences disappeared during the summer and autumn. The strong irradiance in the open field site studied led to a chronic but only mild reduction in F_v/F_m , with values around 0.79. Summer sunflecks led to a sustained photo-inhibition in shade plants, which exhibited a significant reduction in pre-dawn F_v/F_m of 10% with the onset of drought. Photo-inhibition became relatively more important for carbon gain in the shade than in the sun due to the low photochemical efficiency under the low light that follows sunflecks. Sun plants of *H. arbutifolia* exhibited a rather efficient photoprotection against strong irradiance conferred by both the architecture of the crown and the physiology of the leaves. There is evidence that El Niño events and the associated droughts have become more frequent and severe. Counter-intuitively, the effects on plant performance of such extreme droughts could be more critical in the shade than in the sun.

Key-words: *Heteromeles arbutifolia*; chlorophyll fluorescence; drought; light utilization efficiency; Mediterranean-type ecosystems; photo-inhibition; photosynthesis; stomatal conductance; sunflecks; water potential.

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INTRODUCTION

Global change effects on Mediterranean climate are likely to produce warmer and drier conditions, and the transition observed over the last century has been punctuated by severe drought events, exacerbated in certain areas during El Niño Southern Oscillation years (Pavia & Badan 1998; Peñuelas, Lloret & Montoya 2001). Even though plant response to drought is relatively well known, the ecophysiological knowledge required to estimate its implications for plant performance under real field conditions is fragmentary (Cornic 1994; Valentini *et al.* 1995; Valladares & Pearcy 1997; Cornic 2000; Martínez-Ferri *et al.* 2000; Lei 2001). This is due at least in part to the fact that plant response to simultaneous stresses (e.g. drought plus excessive light and heat) is usually not predictable from single-factor studies (Gamon & Pearcy 1990; Valladares & Pearcy 1997).

Mediterranean type ecosystems are characterized by long hot summers, with high irradiance and little or no precipitation, all conditions that strongly limit carbon assimilation and promote photo-inhibition of the photosynthetic apparatus. The interaction between strong irradiance and drought has been investigated in a number of studies aimed at unveiling the processes that lead to photo-inhibition and the mechanisms involved in photoprotection (Brestic *et al.* 1995; Valentini *et al.* 1995; Heber *et al.* 1996). Despite the rather good understanding of the phenomenon of photo-inhibition and its occurrence in natural habitats, the knowledge of its impact on photosynthetic carbon assimilation under field conditions remains scant (Ögren & Rosenqvist 1992; Long, Humphries & Falkowski 1994; Osmond *et al.* 1997; Werner *et al.* 2001). The most likely fate of the photons absorbed in full sunlight and the relative contributions of the different photoprotective mechanisms are relatively well-established (Björkman & Demmig-Adams 1994; Demmig-Adams & Adams 2000; Muraoka *et al.* 2000). The most relevant research now concerns the extent to which these processes are engaged under natural stress conditions (Osmond *et al.* 1997). Furthermore, most of our knowledge on photo-inhibition comes from herbs and annuals, whereas photo-inhibition during the drought period has not been explored adequately in evergreen sclerophylls of

Mediterranean-type ecosystems (Mooney 1989; Osmond *et al.* 1997).

Drought can interact not only with excessive light, but also with limiting light in understory habitats, which has deserved significantly less research. Plant tolerance of combined shade and drought, typically considered irresolvable due to trade-offs in morphology and physiology, potentially determine important vegetation patterns, especially as droughts are becoming more severe world-wide (Sack, Grubb & Marañón 2001). Plant facilitation (i.e. positive plant interactions) in arid environments, which is among other factors casting shade, has been shown to be important for the establishment of key species in Mediterranean-type vegetation (e.g. Pugnaire, Haase & Puigdefábregas 1996; Callaway & Pugnaire 1999). However, both aerial and below ground resource capture in the shade can be severely restricted by intense competition among plants (Abrams & Mostoller 1995; Tielborger & Kadmon 2000).

We have studied leaf water potential, gas exchange and chlorophyll fluorescence field responses to the unusually dry 1994 years in natural sun and shade populations of toyon (*Heteromeles arbutifolia*), an evergreen sclerophyll shrub of the Californian chaparral that exhibits significant phenotypic adjustments to cope with either low or high light (Valladares & Pearcy 1997, 1998, 1999). In addition to the summer drought, California experiences highly abnormal rainfall during El Niño years due to its position as a node in the typical Pacific-North American pattern (Schonher & Nicholson 1989; Pavia & Badan 1998; Jones 2000). Unusually severe droughts took place in the El Niño 1994 years in the Central Valley of California (Fig. 1) as well as in other areas with a Mediterranean type of weather (Peñuelas *et al.* 2001). Our objectives were: (i) to explore the long-term field response of *H. arbutifolia* to the severe 1994 drought in contrasting light environments; (ii) to determine the real efficiency of the structural protection against excessive light provided by the architectural traits of the crown exhibited by this species in open sites (Valladares & Pearcy 1998); and (iii) to explore the physiological effects on the leaves of shade plants of intense and relatively long, naturally occurring sunflecks, especially

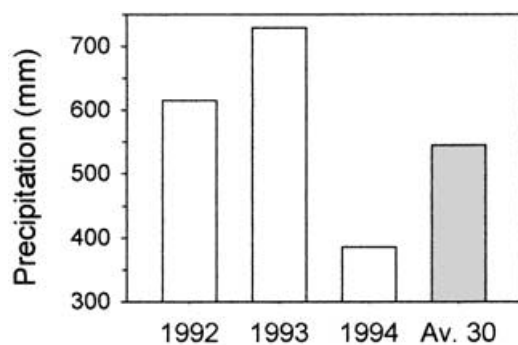


Figure 1. Total annual precipitation registered in 1992, 1993 and 1994, plus the average of the annual precipitation for the last 30 years (Av. 30) at Winters weather station (California, USA).

when combined with drought. We expected not only a more severe effect of drought in the sun than in the shade due to the contrasting evaporative demand, but also a reduced photochemical efficiency (greater photo-inhibition) in the sun as compared to the shade populations of this species. However, the relative importance of photo-inhibition could exhibit the reverse pattern: photo-inhibition may not be a large contributor to photosynthetic losses in water-limited plants under high light due to the overriding effect of stomatal closure (Boyer, Armond & Sharp 1987; Cornic 2000), but it can be important in shade plants because photo-inhibition during sunflecks can significantly affect photosynthetic carbon gain when leaves return to the low, background light of the understory (Pearcy 1994).

MATERIALS AND METHODS

Field site

The populations of toyon (*Heteromeles arbutifolia* M. Roem) studied were located in the G.L. Stebbins Cold Canyon Reserve of the University of California Natural Land and Water Reserve System (38°30' N, 122°06' W). This site is in the Vaca Hills just to the east of the Central Valley of California (USA) and supports a mixture of evergreen chaparral averaging 2–3 m in height interspersed in a 10–15 m tall oak woodland. The chaparral communities are dominated by *H. arbutifolia*, chamize (*Adenostoma fasciculatum*) and *Ceanothus* spp. Overstory trees in this woodland are primarily blue oak (*Quercus douglasii*) and interior live oak (*Quercus wislizenii*). The plants of *H. arbutifolia* studied were located in either a patch of chaparral (sun plants) or an oak woodland understory (shade plants). For more details on the available radiation at each site see Valladares & Pearcy (1998). The oak woodland overstory canopy at this particular site was mostly closed with a leaf area index of about 2 as measured with a LAI-2000 plant canopy analyser (Li-Cor Inc., Lincoln, NB, USA). The region has a strong Mediterranean climate with clear, hot, dry summers and relatively cool and wet winters (Figs 1 & 2). Mean annual rainfall is 540 mm, with considerable year-to-year variations (Fig. 1). In 1994, the year of the study, no precipitation was recorded during the 4-month period of June–September (Fig. 2). Long- and short-term climatic information was obtained from Winters C (CAYOCAB5) weather station (database of the Division of Agriculture and Natural Resources, University of California), which is approximately 5 km east and 40 m lower in elevation than Cold Canyon.

Sampling protocol

Five representative plants in the open chaparral habitat and five in the understory habitat were selected and tagged for repeated measurements at the beginning of 1994. On each plant, 10 healthy fully developed leaves were selected and tagged from the upper, most exposed parts of the shoots of these plants and tagged for subsequent measurements over

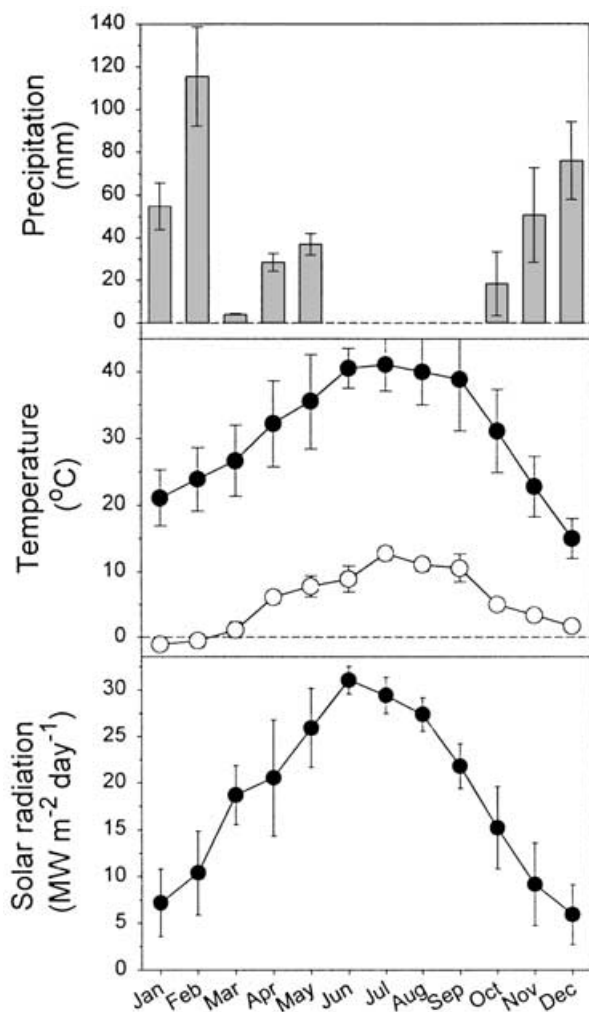


Figure 2. Monthly averages of precipitation, maximum (closed symbols) and minimum (open symbols) air temperature, and solar radiation recorded in 1994 at Winters weather station (California, USA). Error bars represent standard deviation.

the year. On any day during which measurements were taken four leaves on each plant were measured. This sample consisted of a N-, S-, E- and a W-facing leaf to account for orientation effects. An attempt was made to conduct repeated measurements on the same leaves over the year, but when a leaf was lost due to breakage, etc., a comparable one in terms of position and orientation was selected from the remaining marked leaves. Measurements were made at approximately monthly intervals on two consecutive days with clear skies, with the shade plants measured on one day and the sun plants on the other. Complete and comparable data sets were obtained for 11 two-day periods throughout the year.

Leaf water potential

At each sample date, pre-dawn (Ψ_{pd}) and midday leaf water potentials (Ψ_{mid}) were determined with a Scholander

pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA). Water loss from the excised leaves was limited by wrapping them in a small plastic bag prior to excision, and by humidifying the pressure chamber with a wet paper towel. A minimum of 10 leaves (2–3 leaves from five plants) were measured at each time.

Diurnal gas exchange and chlorophyll fluorescence measurements

Daily courses of gas exchange and *in vivo* chlorophyll fluorescence were initiated at pre-dawn and continued at 1–1.5 h intervals until 1 h after sunset. This gave a minimum of eight complete series of measurements for the 20 leaves on each day. Rates of net CO_2 exchange were measured with a portable, closed-system photosynthesis apparatus model Li-6200 (Li-Cor, Inc.). Measurements made before dawn and after sunset were taken as the dark respiration rates (R_{dark}) whereas those made at intervals throughout the day were used to construct daily courses of net photosynthesis (A_{net}). For a measurement, the leaf was clamped inside the cuvette in a way that maintained it in its normal orientation so as to cause minimal disturbance to the light environment, and measurements commenced after a stable rate of decrease in CO_2 concentration was achieved. The system was set to record three successive measurements of photosynthesis with the mean of these three being used as an estimate of A_{net} for a leaf at the sample time. This measurement protocol typically took about 1 min to complete, during which no significant increases in leaf or air temperatures within the chamber were observed. Sampling of all 20 leaves required approximately 30 min. Stomatal conductance to water vapour (g_{wv}) was measured with a portable steady state porometer (Li-1600, Li-Cor, Inc.). Although at most times there was general agreement between the two instruments, the Li-1600 porometer gave more reliable estimates of g_{wv} than the Li-6200 gas analyser especially at the high humidity and low temperature conditions in the morning.

Immediately following the A_{net} and g_{wv} measurements, chlorophyll fluorescence was determined with a portable, pulse-modulated fluorometer (PAM 2000; Heinz Walz, Effeltrich, Germany). Steady-state fluorescence (F) under the prevailing ambient light and maximal fluorescence (F_{m}') during a saturating pulse (approximately 6000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, 800 ms duration) were determined. The quantum yield of PSII (Φ_{PSII}) in the light was calculated from these measurements according to the procedure of Genty, Briantais & Baker (1989). Estimates of the dark-adapted photochemical efficiency of photosystem ($F_{\text{v}}/F_{\text{m}}$) were made at pre-dawn and 1 h after sunset. Leaves were dark adapted with homemade clip holders having a black cylinder that accepted the fluorometer fibre-optic probe and positioned it correctly for the measurement. This holder blocked any ambient light from the leaf both before and during the measurement. For pre-dawn values, the holders were clipped on the leaves the evening before the measurements. For evening measurements, the holders were

attached at sunset and the measurements of F_v/F_m were made 1 h later. Temperature inside the clip holders did not differ from air temperature due to the very low ambient radiation, if any, at the time of measurements, and no differences were detected in F_v/F_m values between pre-dawn values of leaves with the clip holders and those without the holders and measured well before sunrise.

Calculations and statistics

Daily PFD ($\text{mol photons m}^{-2} \text{ d}^{-1}$) and carbon gain ($\text{mmol CO}_2 \text{ m}^{-2} \text{ d}^{-1}$) were integrated from the diurnal courses of incident PFD and A_{net} using the area transformation of SigmaPlot for Windows version 5.0 (SPSS, Chicago, IL, USA).

Apparent electron transport rate through PSII (ETR) was calculated using the following formula of Genty *et al.* (1989):

$$\text{ETR} = 0.82 \times 0.5 \times \text{PED} \times \phi_{\text{psii}} \quad (1)$$

In this equation 0.82 is the leaf absorptance of toyon leaves as measured by Valladares & Pearcy (1998) and 0.5 is the proportion of the photons partitioned to PSII. The fraction of excitation energy dissipated by photorespiration (P_{diss}) was calculated as

$$P_{\text{diss}} = [\text{ETR} - (4 \times A_{\text{gross}})] / \text{ETR} \quad (2)$$

This equation assumes that electrons are utilized either in CO_2 fixation (four per CO_2) or in photorespiration. A_{gross} is $A_{\text{net}} + R_{\text{day}}$, where R_{day} is the respiration rate in the light. To estimate the carboxylation efficiency under natural conditions throughout the year, values of A_{gross} and g_{wv} were taken from leaves naturally exposed to saturating PFD ($> 800 \mu\text{mol m}^{-2} \text{ s}^{-1}$) for a minimum of 10 min prior to measurements both in the open and in the understory (leaves exposed to sunflecks). Intercellular CO_2 concentration (C_i) was calculated according to Eqn 3:

$$C_i = C_a - (1.6 \times A_{\text{net}} / g_{\text{wv}}) \quad (3)$$

where C_a and C_i are the mol fraction of CO_2 in the ambient air and the intercellular air spaces, respectively. The CO_2 compensation point in the absence of non-photorespiratory CO_2 evolution (Γ^*) was initially taken as $37 \mu\text{mol mol}^{-1}$ (mean standard value at 25°C) and corrected for temperature according to the empirical Eqn 4:

$$\Gamma^* = 37 + 0.188 \times (T - 25) + 0.0036 \times (T - 25)^2 \quad (4)$$

where T is leaf temperature in $^\circ\text{C}$. We then calculated a carboxylation efficiency as the slope defined by Γ^* and A_{gross} and C_i for each leaf at each sample time during the day.

The accuracy of the computation of P_{diss} and carboxylation efficiency depends primarily on whether A_{gross} can be estimated from gas exchange measurements. The value Γ^* is apparently invariant among species (Atkin *et al.* 2000) and its temperature dependence is well predicted by Eqn 4. The accuracy of A_{net} and R_{dark} depend on instrumental errors. Since we measured R_{dark} , the value of R_{day} used requires several assumptions. First, mitochondrial respiration has been shown to be inhibited in the light by 16–77%

depending on species and conditions (Atkin *et al.* 2000). Second, R_{day} is sensitive to temperature so that the values obtained at pre-dawn and sunset need to be corrected to temperature at the time of the A_{net} measurement using the empirical equations of Muraoka *et al.* (2000). Finally, however, Atkin *et al.* (2000) have recently shown that the light inhibition of mitochondrial respiration increases with temperature so that R_{day} becomes essentially independent of temperature. We tested how these assumptions influenced the computation of A_{gross} and P_{diss} and found only a 10% difference between the most conservative (no light inhibition, R_{day} predicted by application of the Muraoka *et al.* equations to R_{dark}) to the least conservative (i.e. a high average inhibition of respiration of 60%; $R_{\text{day}} = 0.4 \times R_{\text{dark}}$ at 25°C , no temperature dependence). We concluded that the assumptions chosen would have no significant impact on the conclusions drawn about the seasonal changes or habitat differences and consequently we adopted the most conservative assumptions.

The significance of the differences between open and understory plants and between measurements at different times of the year were tested by ANOVA using Systat for Windows version 9.0 (SPSS, Chicago, IL, USA). Data were curve-fitted by either linear or non-linear regressions with Sigma Plot for Windows version 5.0, which was also used to test for the significance of the fitted function.

RESULTS

Both Ψ_{lpd} and Ψ_{imd} exhibited the expected decline during the dry summer and recovery as rains commenced in the autumn but there were significant differences between the sun and shade plants in the extent of the decline (Fig. 3). Sun and shade plants exhibited similar Ψ_{lpd} values in the spring but the summer decrease in Ψ_{lpd} was much larger ($P < 0.001$) in the understory than in the open habitat. Consequently shade plants had much lower Ψ_{lpd} than sun plants at this time. The diurnal decline in leaf water potential between pre-dawn and midday was larger in sun than shade plants and this caused the sun plants to exhibit lower Ψ_{imd} values than shade plants in the spring (Fig. 3). Indeed during the summer there was almost no diurnal variation in Ψ_l but because the decrease in Ψ_{lpd} was so large Ψ_{imd} was still significantly lower in the understory than in the open habitat. These seasonal patterns are consistent with the development of significantly greater water stress in the shade as compared to the sun plants.

The daily PFD incident on a horizontal surface has been previously reported to be 7 times higher in the sun than the shade habitat (i.e. 54 versus $7.7 \text{ mol m}^{-2} \text{ d}^{-1}$ in a clear summer day, Valladares & Pearcy 1998). The leaves however, were not necessarily exposed to this level of PFD because of leaf orientation effects. In the open habitat, PFDs during the measurements were typically $700\text{--}1100 \mu\text{mol m}^{-2} \text{ s}^{-1}$ because of the steeply inclined leaf surfaces. This PFD was close to the level required to light saturate photosynthesis at least in the spring when assimilation rates were relatively

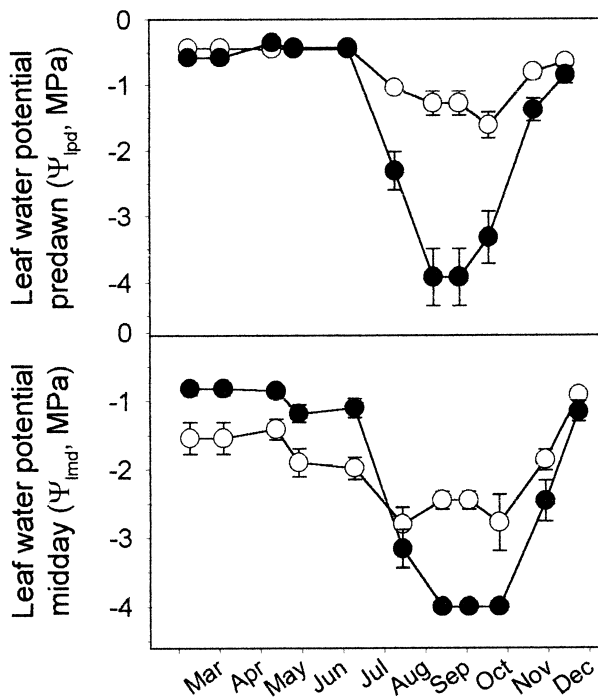


Figure 3. Seasonal evolution of pre-dawn and midday leaf water potential in sun (open symbols) and shade (closed symbols) plants of *H. arbutifolia* during 1994. Values are the mean of 20 leaves (four leaves of different orientations from five different plants) \pm standard deviation.

high, indicating that the leaves received relatively little PFD in excess of the level that can be utilized for increasing assimilation. Leaves in the understory habitat were generally oriented much closer to horizontal and consequently they received PFDs in the range of 1200–2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during the relatively long sunflecks (from 20 min up to 1 h).

Daily carbon gain was significantly higher ($P < 0.01$) throughout the year in sun than in shade plants due to the combined effect in the sun of a higher photosynthetic capacity and a higher available PFD (Fig. 4). The value of g_{wv} at midday was also significantly higher ($P < 0.01$) in the open habitat than in the understory most of the year (Fig. 4). Both daily carbon gain and midday g_{wv} exhibited a strong decrease during the summer as leaf water potentials declined. By August and September, when drought stress was most severe, the leaves in the understory habitat barely exhibited a positive daily carbon gain and had very small stomatal conductances. Although drought stress had obvious and severe effects on daily carbon gain, there was little evidence that this predisposed the leaves to severe photo-inhibition. Pre-dawn F_v/F_m values were significantly lower ($P < 0.001$) in the sun than in the shade plants in the spring, suggesting some chronic photo-inhibition in the former, but they also remained essentially unchanged in sun plants during the development of severe water stress in summer (Fig. 5). For shade plants, F_v/F_m did decrease during the summer but only to the level exhibited by the sun plants. A similar

seasonal trend was observed in the quantum yield of PSII (Φ_{PSII}) at midday, although in this case autumn values exhibited a clear recovery (Fig. 5). Thus, even in these leaves that underwent severe drought-induced reductions in carbon gain and experienced long bright sunflecks, there was evidence for no more than mild chronic photo-inhibition. The decline in F_v/F_m that was apparent in both habitats in November occurred when the measurements were made following a period of low night temperatures.

Photorespiratory dissipation (estimated according to Eqn 2) at midday was significantly higher ($P < 0.05$) in the sun than in the shade in spring and autumn, but not during the summer due to its significant ($P < 0.01$) increase in shade plants during this season (Fig. 5).

We examined the relationships between A_{net} , g_{wv} , ETR and PFD as measured during the gas exchange determinations after segregating the data according to habitat and season and plotting assimilation rate against the other variables (Fig. 6). We take the upper boundaries formed by the data points in these plots to indicate the relationships between the two respective variables when other factors are not limiting. Data points falling below these boundaries on the other hand are taken to indicate the presence of additional unspecified limitations on assimilation rate. As expected, values from the shade habitat were mostly at the lower left because most measurements were in low PFD whereas those from the open habitat were predominately

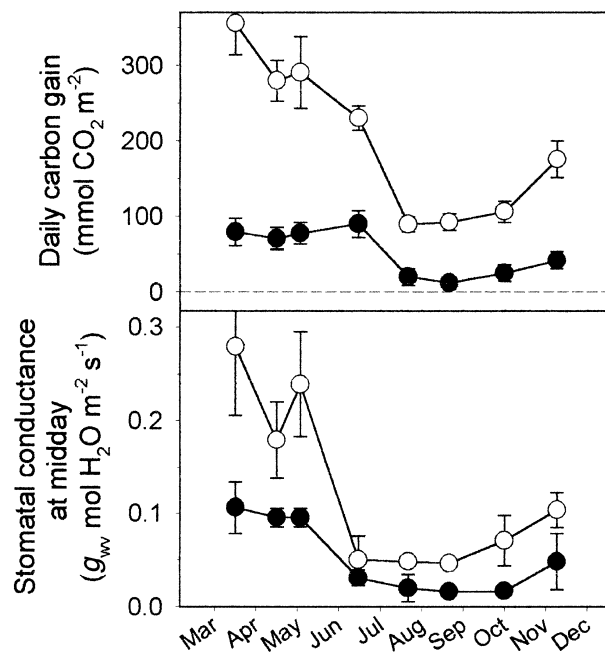


Figure 4. Seasonal course of daily carbon gain and stomatal conductance to water vapour at midday in sun (open symbols) and shade (closed symbols) plants of *H. arbutifolia*. Midday values in shade plants were taken when leaves were under diffuse PFD (i.e. not under a sunfleck). Values are the mean of 20 leaves (four leaves of different orientations from five different plants) \pm standard deviation.

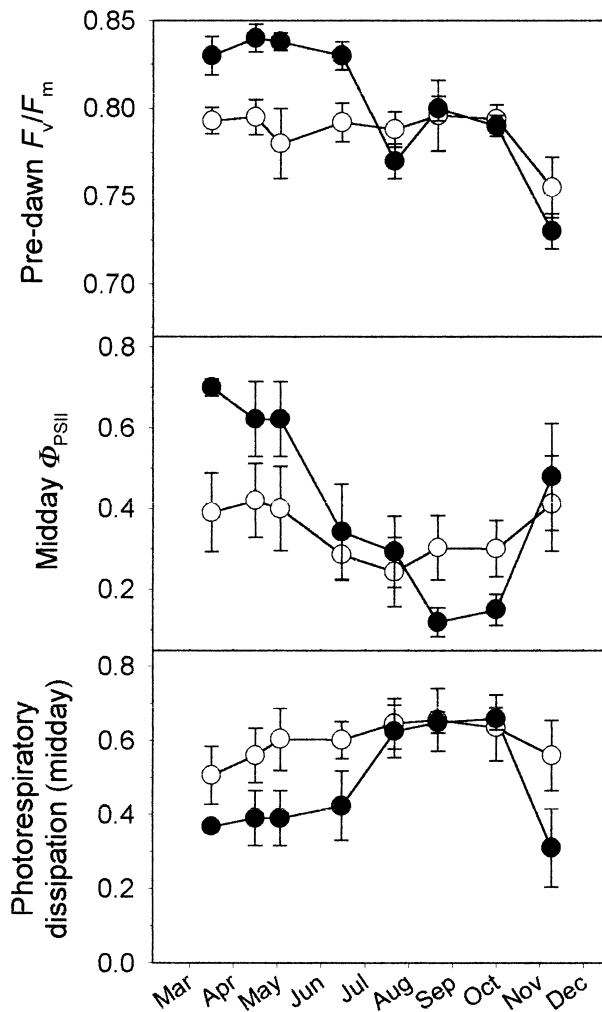


Figure 5. Seasonal evolution of pre-dawn photochemical efficiency (F_v/F_m), midday quantum yield of photosystem II (Φ_{PSII}), and midday photorespiratory dissipation [estimated as $(ETR - 4A)/ETR$, where ETR = electron transport rate estimated by *in vivo* chlorophyll fluorescence, A = gross photosynthesis, see Material and Methods] in sun (open symbols) and shade (closed symbols) plants of *H. arbutifolia* during 1994. Midday values in shade plants were taken when leaves were under background PFD (i.e. not under a sunfleck). Values are the mean of 20 leaves (four leaves of different orientations from five different plants) ± 1 standard deviation.

dispersed to the upper right. For the open habitat, comparisons revealed the higher photosynthetic rates at high PFD in the spring as compared with the summer (Fig. 6). At low PFDs, little difference was apparent in A_{net} for either the different habitats or the seasons. The upper bound between A_{net} and g_{wv} was somewhat steeper in the summer and autumn as compared to the spring. This gave rise to higher A_{net} at lower g_{wv} in the summer causing generally lower C_i values during this period of maximum drought stress (Fig. 7). In the spring, g_{wv} values were often high relative to A_{net} giving a wide dispersion to the relationship between A_{net} and g_{wv} . In the shade habitat, C_i values remained high throughout the year principally because most measure-

ments were under light-limiting conditions for photosynthesis (Fig. 7).

ETR varied in a positive manner with both PFD and with A_{net} but seasonal differences were apparent. The range of ETR values was similar in the spring and summer but because of the lower A_{net} values in the summer, ETR tended to be lower for any given value of A_{net} . In the autumn, the range of ETR values was reduced in comparison with the summer because the maximum PFDs were lower, but otherwise the relationship between A_{max} and ETR was similar to that observed in the summer. There was no autumn recovery to the high values of ETR observed in the spring. This is further shown in the seasonal progressions of ETR (Fig. 8) from high values in the spring followed by a nearly 50% reduction in the summer and autumn. In contrast, ETR values of the shade plants exhibited little seasonal variation.

Calculation of the carboxylation efficiencies revealed that, in addition to the limitation on A_{max} imposed by the strongly decreased g_{wv} in midsummer, a further limitation due to a decrease in carboxylation efficiency also developed (Fig. 8). The absolute decrease from early spring to midsummer and the recovery in the autumn was greater in the sun than in the shade but, because of the overall lower values in the understory in comparison with the open, the relative changes over the year were similar in the two habitats. Since carboxylation efficiency is mostly a function of the biochemical capacity for net CO_2 assimilation in the mesophyll, it indicates a constraint on these processes by water stress and/or the higher temperatures in midsummer in comparison with the spring and autumn.

Daily photon use efficiencies also exhibited large seasonal changes related to the drought cycle (Fig. 8). In the spring, photon use efficiencies were higher in the understory than in the open habitat but as the drought developed this pattern reversed so that they were slightly higher in the open habitat. Photon use efficiencies calculated for sunflecks exhibited an even stronger decrease from high values in the spring to nearly zero in midsummer. This indicates that constraints on sunfleck utilization imposed by stomatal and carboxylation limitations essentially prevented their utilization for significant carbon gain in the summer with little recovery in the autumn. In contrast utilization of sunflecks, which contributed 45–55% of the available PFD in the understory, accounted for 65–75% of the carbon gain in the March to May period when carbon gain was highest.

DISCUSSION

Differential effects of a severe drought in the sun and in the shade

Despite the higher evaporative demand in the sun, the plant water potential reductions during the dry months were more severe in the understory than in the open habitat (Fig. 3). The much lower Ψ_{pd} is consistent with a greater soil moisture depletion in the understory in comparison with the open site due to greater competition for water in the

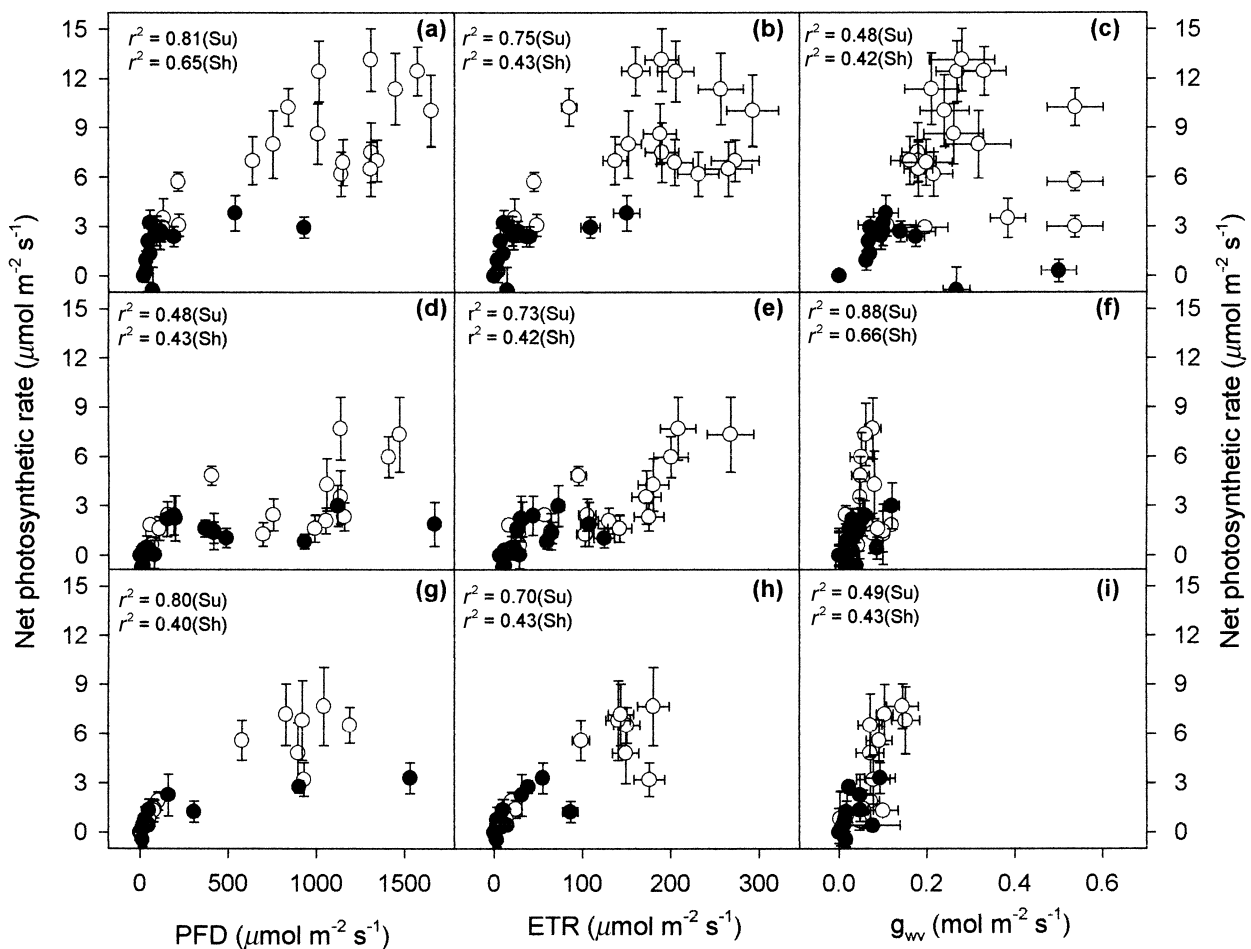


Figure 6. Influence of irradiance (PFD, graphs a, d and g), electron transport rate (ETR, estimated by *in vivo* chlorophyll fluorescence, graphs b, e, h) and stomatal conductance to water vapour (g_{wv} , graphs c, f and i) on net photosynthesis in sun (open symbols) and shade (closed symbols) plants of *H. arbutifolia* during the spring (graphs a, b and c), summer (graphs d, e and f), and autumn (graphs g, h and i). Values are the mean of 20 leaves (four leaves of different orientations from five different plants) \pm standard deviation. Coefficients (r^2) for non-linear regression (hyperbolic function) are given for each case.

former. Similar results have been reported for tree seedlings in a more humid, temperate forest (Abrams & Mostoller 1995). *Heteromeles arbutifolia* is a relatively shallow-rooted shrub (Davis & Mooney 1986). Moreover, shade adaptation typically involves a decreased root : shoot ratio possibly further restricting the competitive ability for water acquisition. Shade plants also exhibited a greater decrease in g_{wv} , A_{net} , and daily carbon gain in response to drought than sun plants (Fig. 4). The increased opportunity for sun plants to rehydrate overnight might be responsible for their ability to maintain higher relative gas exchange rates than shade plants during the dry months.

The physiological response of plants to either sun or shade in combination with drought differ among species and habitats. Muraoka *et al.* (1997) found that stomatal conductance and photosynthetic rates of the herb *Arisaema heterophyllum* on humid floodplains in Japan were lower in open sites than in the understory, which agrees with the photoprotection and facilitation attributed to nurse plants in dry areas (Callaway & Pugnaire 1999; Holmgren 2000).

In contrast the reverse was true for *H. arbutifolia* studied here and for tree seedlings in an eastern North-American deciduous forest (Abrams & Mostoller 1995). Combined shade and drought imposes special constraints, because mechanisms for capture of aerial resources such as increased investment in leaf area restricts investment for the capture of below-ground resources (Sack *et al.* 2001). Probably the only way to avoid this conflict is to develop a reduced resource demand, which is characteristic of the stress-tolerator syndrome in general (Grubb 1998) and of Mediterranean woody plants in particular (Valladares *et al.* 2000).

Mild photo-inhibition is relatively frequent in natural vegetation, even under conditions that are optimal for photosynthesis or at light levels that are below light saturation (Ögren 1994). The strong irradiance in the open field site studied here (typically 45–55 mol photon $\text{m}^{-2} \text{d}^{-1}$ in summer) led to a chronic but only mild reduction in pre-dawn F_v/F_m . This small chronic reduction was evident in the small difference in F_v/F_m between the understory and open hab-

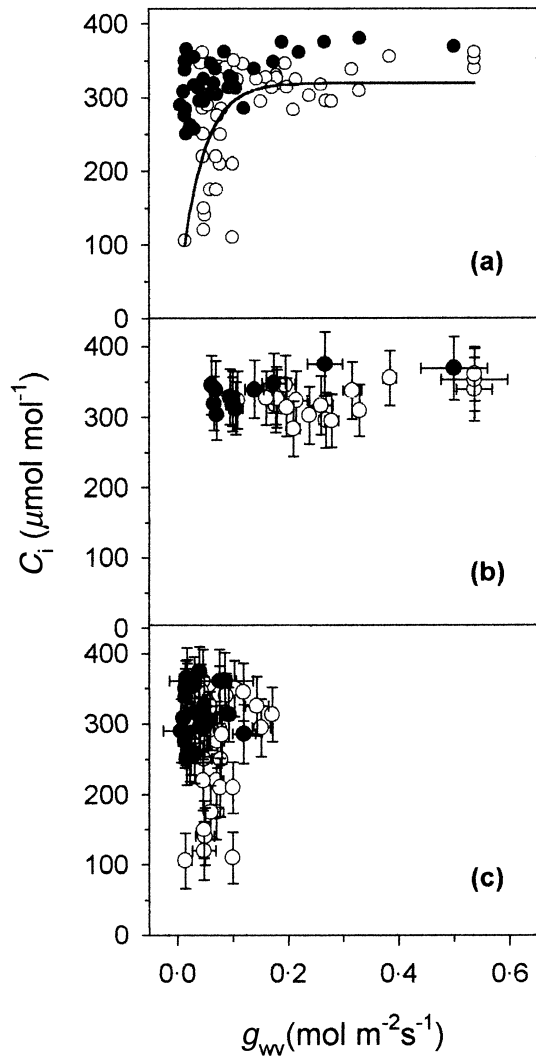


Figure 7. Influence of stomatal conductance to water vapour (g_{wv}) on intercellular CO₂ concentration (C_i) in sun (open symbols) and shade (closed symbols) plants of *H. arbutifolia*. All data are shown in (a), while only spring data are shown in (b), and summer plus autumn data are shown in (c). The regression line ($P < 0.001$) in (a) is for all data of sun plants [$r^2 = 0.56$, $C_i = 319.6 (1 - e^{-25.1g_{wv}})$]. No significant regression was found for shade plants. Values are the mean of 20 leaves (four leaves of different orientations from five different plants) ± 1 standard deviation, except in (a), where error bars have been removed for clarity.

itat plants in the spring. However, the development of drought stress coupled with the higher temperatures during the summer led to no further reductions in F_v/F_m in the open habitat. This indicates a relatively mild photo-inhibition of *H. arbutifolia* in open field sites even under conditions where it might be expected to be exacerbated by the co-occurrence of other stresses. In a previous study with potted plants (Valladares & Pearcy 1997), we have shown that strong photo-inhibition can be observed in this species when leaf angles are manipulated so that the surfaces are exposed to higher PFDs and leaf temperatures than those

experienced by leaves in their normal orientation. Clearly, the leaves on these field plants in the open habitat did not reach these conditions or were more resistant to them.

In agreement with a field study of willow (Ögren & Sjöström 1990; Ögren 1994), the mild photo-inhibition of *H. arbutifolia* probably only minimally impacted total carbon gain. The reduction in F_v/F_m would be expected to reduce

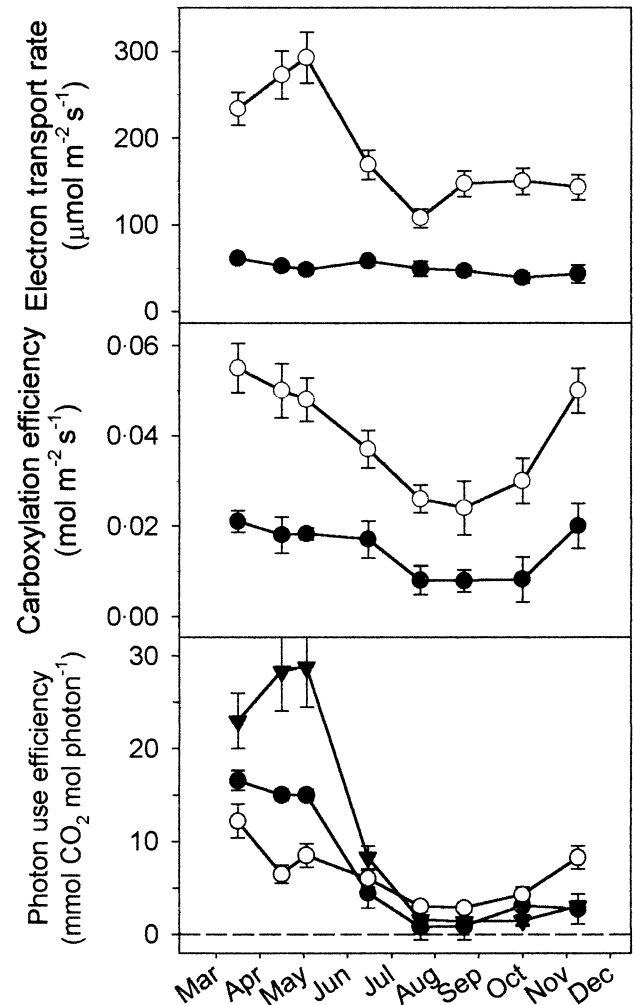


Figure 8. Seasonal course of (a) carboxylation efficiency (b) electron transport rate and (c) photosynthetic photon use efficiency (carbon gain per incident PFD integrated for the whole day) in sun plants (open symbols) and in shade plants (closed symbols) of *H. arbutifolia*. Electron transport rate of shade plants was measured during sunflecks during which the leaves were exposed to saturating PFD ($> 800 \mu\text{mol m}^{-2} \text{s}^{-1}$) for at least 10 min. Photosynthetic photon use efficiency of shade plants is shown both for the whole day (circles) and for saturating sunflecks (triangles) only obtained by integrating the PFD and carbon gain under the curves. Sunfleck data for shade plants were obtained from leaves exposed to sunflecks with saturating PFD ($> 800 \text{mmol m}^{-2} \text{s}^{-1}$) for a minimum of 10 min during the morning peak of photosynthesis. Sunflecks took place typically between 1000 and 1300 h local time and lasted for 20 min on average. Values are the mean of 20 leaves (four leaves of different orientations from five different plants) ± 1 standard deviation.

assimilation in the light-dependent part of the light response curve and in the region of curvature from light-limited to light-saturated photosynthesis. This limitation would be important principally in the self-shaded parts of the crown, especially as leaves transition from direct sunlight to shade due to the diurnal change in solar position in the sky. It would however, become even less important in the summer as stomatal closure and reductions in carboxylation efficiency cause the light saturation point of photosynthesis to decrease. The apparently chronic reduction in F_v/F_m may reflect a trade-off of long-term acclimation to mean light conditions of leaves with moderately high photosynthetic capacities rather than a damage resulting from insufficient acclimation. These sustained but moderately low F_v/F_m values suggest photoprotection by the xanthophyll cycle pool rather than photodamage involving the turnover of proteins D1 and D2 (Demmig-Adams & Adams 2000; Ort 2001). Our results support the idea illustrated elsewhere (Valladares & Pearcy 1998, 1999) that the steep foliage and relatively short internodes leading to considerable self shading that is characteristic of the sun individuals of this species are efficient in minimizing photo-inhibition. This finding emphasizes the importance of considering crown architecture along with leaf physiology studies of photo-inhibition and long-term carbon gain under field conditions (Valladares & Pearcy 2000; Werner *et al.* 2001).

Light can be excessive not only in the open, but at least transiently excessive also in the understory, because the background, diffuse light is punctuated with sunflecks of varying duration and PFD. These sunflecks can lead to photo-inhibition of shade-adapted leaves (Pearcy 1994). In fact, leaves of the shade plants studied here were exposed to significantly higher PFDs during sunflecks (typically $>1450 \mu\text{mol m}^{-2} \text{s}^{-1}$) than leaves in the sun plants at any time of the day (typically $<1250 \mu\text{mol m}^{-2} \text{s}^{-1}$). This was due to the almost horizontal leaf arrangement and minimal self-shading of the shade plants of *H. arbutifolia* (Valladares & Pearcy 1998). Additionally, because photosynthetic capacities were lower in shade than in sun leaves, and drought constrained photosynthetic rates to a much greater extent in the former, PFD was even more excessive in the shade than the sun. Indeed in the shade a sustained photo-inhibition was evident in that pre-dawn F_v/F_m was reduced by 10% at the onset of drought. This was in contrast with sun plants, which exhibited no further reduction in pre-dawn F_v/F_m during the dry months. Sunflecks contributed more than 50% of the total PFD available for the understory plants and were rather efficiently used by shade *H. arbutifolia* leaves to fix carbon during the spring. During the summer, however, utilization of sunflecks was strongly constrained to the point that they did not render any noticeable net carbon gain during the driest months.

Structural photoprotection, such as that observed in sun plants of *H. arbutifolia*, not only minimized photo-inhibition but also leaf overheating. Overheating can also be avoided by transpirational cooling, but this may lead to severe water deficits and cannot be afforded under extreme drought (Matsumoto, Muraoka & Washitani 2000). Leaf

overheating under water deficit, heat and strong irradiance has been shown to lead to leaf necrosis in *H. arbutifolia* when the photoprotection provided by the steep leaf angles was artificially removed by reorienting leaves (Valladares & Pearcy 1997).

Mechanisms involved in the reduction of carbon gain with water stress

There is ample documentation on the importance of stomatal closure in photosynthesis suppression under dry and high light conditions, but stomatal limitation has rarely been quantitatively evaluated under natural conditions (Valentini *et al.* 1995; Tezara *et al.* 1998; Muraoka *et al.* 2000). In our study, the value of g_{wv} was significantly reduced with the onset of drought both in the sun and in the shade, especially at midday (Fig. 4). This reduced g_{wv} led to a sharp decrease of net photosynthesis both in the sun and in the shade. The values of g_{wv} observed in *H. arbutifolia* sun plants during the summer field conditions were less than half of those measured in well-watered plants under similar environmental conditions during the same year (Valladares & Pearcy 1997); daily carbon gain was one-third of that of well-watered plants from the same experiment. The decrease in C_i with stomatal closure was minimal in both light environments (Fig. 7). Such a pattern is consistent with a concomitant reduction of carboxylation efficiency (Fig. 8). Tezara *et al.* (1998) found a similar pattern and similar values of carboxylation efficiency under drought in C_3 shrubs from a semi-arid ecosystem. This indicates that mesophyll limitations were also increased with drought in the plants studied and that they were at least in part responsible for the reduced carbon gain during the dry period. However, this interpretation must be accepted cautiously because if stomatal conductance is patchy over the leaf surface, c_i can be overestimated in heterobaric leaves (Terashima 1992) if cuticular transpiration is high (Kirschbaum & Pearcy 1988). Neither of these conditions are likely to significantly alter the conclusions drawn here however. Cornic (2000) concludes that, stomatal patchiness should not be an important problem in the estimations of biochemical versus stomatal limitations to gas exchange under drought stress. Cuticular transpiration, which could induce an error of 10% or less (Muraoka *et al.* 2000) should be minimal in *H. arbutifolia* due to its thick, presumably rather water-tight cuticle.

During the driest months, even the plants in the shade exhibited a relatively high electron transport rate that was clearly not related to the photochemical carbon reduction sink of photosynthesis (Fig. 8). This agrees with the observations of large residual electron transport capacity in the extremely drought-stressed leaves of madrone (*Arbutus menziesii*) in California (O. Björkman, unpublished results discussed in Osmond *et al.* 1997). In that case, the electron transport could not be fully related to the thermal sink, the dominant fate of absorbed photons during water stress in full sunlight (Björkman & Demmig-Adams 1994), so Osmond *et al.* (1997) argued that a potential additional sink could involve photorespiration and photoreduction of O_2 in

the Mehler-ascorbate peroxidase pathway. The capacities of photorespiration and the Mehler pathway to mitigate photo-inactivation of photosystem II have been clearly demonstrated (Wingler *et al.* 1999), but even though more research is needed to quantify their relative contributions to dissipating excess light energy, it seems that neither can alone prevent photo-inhibition in strong light (Osmond *et al.* 1997). Photorespiration has been shown to exert a potentially protective role against combined stresses such as drought in high light (Valentini *et al.* 1995; Wingler *et al.* 1999, 2000; Muraoka *et al.* 2000). Valentini *et al.* (1995) estimated that carbon loss by photorespiration reached 56% of net assimilation rates in water-stressed leaves of Turkey oak (*Quercus cerris*), which agrees with the values obtained here with our simple estimation of photorespiratory dissipation (Fig. 5). Muraoka *et al.* (2000) found larger rates of photorespiration in sun leaves than in shade leaves of an Asian floodplain herb, which also agrees with our estimates. Sun and shade plants of *H. arbutifolia* exhibited similar values of photorespiratory dissipation expressed as a fraction, but absolute values were larger in the sun.

CONCLUSIONS

Establishment of plants in arid environments is frequently dependent on favourable conditions provided by other plants, partly because of the shade (Callaway & Pugnaire 1999). However, we have shown here that the capacity to withstand a severe drought is not only not enhanced in the shade but decreased, probably due to increased below ground competition for water with established trees. Photo-inhibition became relatively more important for carbon gain in the shade than in the sun due to the low photochemical efficiency under the low light that follows sunflecks. Sun plants of *H. arbutifolia* exhibited a rather efficient photo-protection against strong irradiance conferred by both the architecture of the crown and the physiology of the leaves. There is increasing evidence that El Niño (ENSO) events and the droughts associated with them have become more frequent and more severe in recent decades, which reduces the capacity of forest ecosystems to recover before the next drought (Peñuelas *et al.* 2001; Harrison 2001). Counter-intuitively, the effects on plant performance of such extreme droughts could be more critical in the shade than in the sun.

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