Photoinhibition of photosynthesis and photorespiration in *Vitis vinifera* under field conditions — effects of light climate and leaf position

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Abstract

Field-grown grapevines (*Vitis vinifera* cv. Cabernet Sauvignon) were examined for photoinhibition under field conditions. Attached leaves at different positions along the shoot were investigated and their net-assimilation, photorespiration and electron transport were measured. The photochemical efficiency of photosystem II was evaluated on detached, dark-adapted leaves by determining differences in chlorophyll fluorescence, using a portable fluorometer. The comparison of unshaded and artificially shaded plants allowed an estimate of direct photoinhibitory effects on their photochemical capacity. Photoinhibition was also quantified for dark-adapted leaves following exposure to moderate and high light. The immature apical leaves had a lower assimilation rate than mature leaves. This was attributable in part to greater photoinhibition in immature leaves due to greater non-photochemical quenching of fluorescence (q_i) and commensurate inhibition of the photochemical efficiency of system II of photosynthesis (F_v/F_m). This inhibition coincided with the high levels of solar radiation at noon but became less during the afternoon. Shade-adapted leaves were more sensitive to photoinhibition than sun-adapted leaves but light acclimatisation effects were independent of photochemical quenching, as distinct from non-photochemical quenching. The degree of photoinhibition was inversely related to photochemical quenching under both low and high light. Practical implication of fluorescence measurements are discussed.

Abbreviations and Symbols

$\mathbf{A_r}$ assimilation rate; $\mathbf{C_i}$ interior partial pressure of CO_2; **ETR** apparent electron transport rate; $\mathbf{F_0}$ minimal fluorescence (dark), all reaction centres of PSII open; $\mathbf{F_m}$ maximal fluorescence (dark), all reaction centres of PSII closed; $\mathbf{F'_m}$ maximal fluorescence (light), $\mathbf{F_t}$ transient fluorescence; $\mathbf{F_v}$ variable fluorescence (dark), $F_v = F_m - F_0$; $\mathbf{F_v/F_m}$ maximum quantum yield of PSII photochemistry; $\mathbf{J_a}$ electron transport rate calculated from gas exchange data; **PH** photorespiration rate; **PPFD** photosynthetic photon flux density; **PSII** photosystem II; Φ oxygenation/carboxylation ratio; $\mathbf{q_P}$ photochemical quenching; $\mathbf{q_N}$, $\mathbf{q_i}$ total and photoinhibitory non-photochemical quenching of variable fluorescence; $\mathbf{q_I}$ q_N after 15 min of dark relaxation; $\mathbf{R_d}$ day respiration rate; $\mathbf{R_n}$ night respiration rate; $\mathbf{T_{leaf}}$ leaf temperature; $\Gamma*$ CO_2 compensation point in the absence of respiration; $\mathbf{v_o}$ rate of oxygenation.

Introduction

Diurnal and seasonal changes of light intensities in the field range from near darkness to levels well in excess of what is required for maximum photosynthesis. Excess light has the potential to damage the photosynthetic apparatus and plants have evolved strategies to minimise its detrimental effects. For example, some plants move their leaves or chloroplasts away from the sun so as to reduce the light-absorbing area and radiation load (Hirata et al. 1983). Others have evolved metabolic processes that inactivate excess light energy and thus protect the photosynthetic apparatus from permanent damage. It has been suggested that photoinhibition and photorespiration are such processes (Öquist et al. 1992, Ögren and Rosenqvist 1992).

Photoinhibition occurs when the primary photochemical system of photosystem II is inactivated by high light, resulting in loss of photosynthetic capacity manifested as a reduction of quantum yield (Powles 1984). Environmental stress conditions, such as extremes of temperature, water deficits or osmotic stress, may predispose plants to photoinhibition and exacerbate its detrimental effects on net photosynthesis (Powles et al. 1983, Schreiber and Berry 1977, Ludlow and Powles 1988). However, even plants not suffering from any apparent stress were shown to be susceptible to photoinhibition in the field (Ögren 1988). Leaves that have developed in the shade are normally more sensitive to photoinhibition from bright light than leaves that developed in the sun. This

greater sensitivity is often attributed to their larger light-absorbing chlorophyll antenna for photosystem II and lower rates of light-saturated photosynthesis (Öquist et al. 1992).

Characteristic changes in the pattern of chlorophyll fluorescence have been used widely to assess the degree of photoinhibition (Krause and Weis 1984), but its measurement traditionally has been restricted to the laboratory. Recent advances in instrumentation have provided a non-destructive method for rapid assessment of chlorophyll fluorescence in the field (Schreiber et al. 1994). In addition to the traditional analysis of fluorescence patterns these methods allow an assessment of quenching coefficients providing information on *in vivo* photosynthesis.

Photorespiration, as distinct from photoinhibition, is the light-dependent evolution of CO_2 in C_3 plants which derives from glycine oxidation and causes a reduction in the rate of photosynthetic CO_2 assimilation (Zelitch 1979). Photorespiration, similar to photoinhibition of photosynthesis, involves the dissipation of light energy. Therefore, both processes may be considered as distinct mechanisms with the potential to protect the leaf from damage due to excess light. Because both mechanisms involve the dissipation of energy they also influence the efficiency of the photosynthetic process.

To our knowledge, the importance of photoinhibition and photorespiration in grapevines (*Vitis vinifera*) has not been evaluated to date in the field. There is evidence from pot experiments that *Vitis vinifera* (Downton 1983) and the related wild grape *Vitis californica* (Gamon and Pearcy 1990) are susceptible to photoinhibition when stressed by water shortage and high temperature. As far as we know only Düring (1988) estimated the rate of photorespiration in *Vitis vinifera* in the field, showing that photorespiration increased from 35–52% under conditions of water stress.

The aim of this paper was to estimate the diurnal course of photorespiration and photoinhibition of *Vitis vinifera* in the field throughout a growing season. The role of leaf age, related to leaf position along the shoot, and the level of irradiance were also considered. The contribution of PH and PI to net assimilation rate in response to the level of irradiance were assessed by estimating the rate of electron transport from gas exchange and modulated fluorescence measurements. We also aimed to provide information how measurements of modulated chlorophyll fluorescence could be applied to grapevines in the field.

Materials and methods

Plant material

Vines of cv. Cabernet Sauvignon growing in two rows in an experimental vineyard of the CSIRO Division of Horticulture near Mildura (Victoria, Australia) and pruned to canes or by minimal pruning (Clingeleffer 1989) were used in this work. The vines in one row were left without shades while those in the other row were covered after berry set (E-L stage 27, Coombe 1995), on 20 November, with 3 m–wide shade cloth. This reduced the incident radiation by about 50% without significantly modifying other environmental variables. The effects of pruning did not cause statistically significant differences in the behaviour of cane- and minimal pruned vines. Therefore, results will be reported without differentiation between the pruning treatments.

Two experiments were done, both comprising field and laboratory procedures. In experiment 1, four shaded and four unshaded plants were selected and on each plant four well-exposed shoots carrying one bunch each were marked. On each of these shoots, three leaves, in a basal (fully developed but not yet senescent), medium (fully developed) and apical position (not yet fully developed) were selected for measurement of gas exchange and fluorescence yield. In experiment 2, the same number of vines and shoots as in experiment 1 were selected but only two leaves per shoot from a medium position (fully developed) were assessed.

In both experiments, attached leaves were used for field measurements of gas exchange and fluorescence yield. For the laboratory assays, two discs (2 cm^2 diameter) were punched from each leaf previously assessed in the field. Discs were kept hydrated on damp filter paper and were dark-adapted for 30 min before use.

Treatments

Measurements were made in experiment 1 on day A, 10 days after the vines were shaded, and in experiment 2 on that day and three other days (days B, C and D) during the period December to February 1992/93. Weather conditions were variable on day A (1 December), overcast on day B (11 December) and sunny on days C and D (5 January and 12 February). On each sampling day, either photosynthesis or dark respiration and fluorescence were assayed four times, before sunrise at 05.00, (run 1), at 09.00 (run 2), 13.00 (run 3) and 17.00 (run 4). The same leaves were assessed on each run. Water potential was determined on all days except the first, day A, by assessing *in situ* three exposed leaves per vine, using a pressure bomb.

Measurement of fluorescence

In both field and laboratory studies, the fluorescence of chlorophyll was measured with a modulation fluorometer (PAM 2000, Walz, Effeltrich, Germany). The unit was connected to a microcomputer (DEC, PC-333) for data acquisition, data storage and software control (DA-2000, Ver. 1.0; Walz, Effeltrich, Germany).

In the field, fluorescence was assayed on light-adapted leaves. A measuring beam (0.02 µmol/(m^2.s) and a single flash of 3400 µmol/(m^2.s) of 0.2 s duration were applied. The quantum efficiency of PSII was assessed according to the protocol described by Genty et al. (1989) using the nomenclature of van Kooten and Snel (1990). Quantum efficiency of photosystem II was calculated as:

$$\text{Yield} = (F'_m - F_t) / F'_m$$

where F'_m is maximal fluorescence, F_t is transient

fluorescence and the prime sign refers to a state of light adaptation. After each measurement cycle in the field, two discs were punched from each sampled leaf and dark-adapted for at least 30 min in the laboratory before determination of their maximal (F_m), minimal (F_0) and variable ($F_v = F_m - F_0$) fluorescence. These values were used to calculate the maximal quantum yield of photosystem II:

$$F_v / F_m = (F_m - F_0) / F_m$$

In addition, an estimate of photochemical (q_P) and non-photochemical quenching (q_N) was obtained for the midday sampling (13.00). After dark adaptation, discs from shade- and sun-adapted leaves were given a 500 µmol/(m^2.s) (low) and 1500 µmol/(m^2.s) (high) photoinhibition treatment. The high-light treatment was achieved with a halogen lamp directed toward the adaxial surface of the leaf discs. The low-light treatment was achieved by wrapping the discs in white muslin prior to exposure. Leaf discs were maintained well-hydrated on damp filter paper at 25°C and flushed with a stream of air containing 400 ppm CO_2 prior to the light treatment. After 30 min of exposure, the actinic light source was switched off. Henceforth, during a 20 min period of dark relaxation, fluorescence was measured every 2 min, using flashes of 1 s. This frequency is sufficiently low to avoid any significant influence of flashes on the dark relaxation (Quick and Stitt 1989). A far-red beam was used to measure the transient fluorescence minimum (F'_0) corresponding to a state where all PSII traps are re-opened. The photochemical and non-photochemical quenching q_P and q_N were calculated as:

$$q_P = (F'_m - F_t) / (F'_m - F'_0)$$
$$q_N = 1 - (F_m - F'_0) / (F_m - F_0)$$

After 15 min of dark relaxation the ratio F_v/F_m was compared with that prior to applying the photoinhibitory treatment. The ratio was used as an estimate of the degree of photoinhibition (per cent inhibition of F_v/F_m; Horton and Hague 1988, Ögren and Rosenqvist 1992). An estimate of photoinhibition was also obtained by calculating q_I (q_N after 15 min of dark relaxation) as described by Horton and Hague (1988).

Measurements of gas exchange

Gas exchange of CO_2 and H_2O was measured in the field using a portable closed photosynthesis system (Li-COR 6200, Lincoln, Nebraska, USA). The PPFD for each leaf was recorded by placing a quantum sensor (Li-COR LI-190SA) parallel to the leaf surface.

The rate of photorespiration was assessed according to Sharkey (1988):

$$2PH = v_o = \frac{(A_r + R_d)}{\left(\frac{1}{\Phi} - 0.5\right)}$$

where PH is rate of photorespiration, v_o is rate of oxygenation, A_r is assimilation rate, R_d is rate of day respiration and Φ is oxygenation/carboxylation ratio. R_d was calculated from measurements of the night respiration rates according to Kirschbaum and Farquhar (1987) as:

$$R_d = R_n \cdot 0.4$$

where R_n is night respiration. The R_d values were corrected for the temperature difference between the light and dark period, using a value of 71 kJ/mole for the activation energy (Hall 1979).

The ratio of oxygenation/carboxylation (Φ) was calculated according to Farquhar et al. (1980):

$$\Phi = \frac{2 \cdot \Gamma*}{C_1}$$

where $\Gamma*$ is the compensation point in the absence of respiration and C_i the interior partial pressure of CO_2. A value for $\Gamma*$ was calculated as follows

$$\Gamma* = (42.7 + 1.68 \cdot (T_{leaf} - 25) + 0.012 \cdot (T_{leaf} - 25)^2) \cdot P$$

where T_{leaf} is leaf temperature and P is air pressure (Brooks and Farquhar 1985).

In order to estimate photoinhibition from gas exchange data, the influence of photorespiration on CO_2 assimilation rate was removed by calculating the rate of total electron transport (J_a) at the actual leaf temperature. The procedure was outlined by Ögren and Rosenqvist (1992) using the model proposed by Farquhar and von Caemmerer (1982) and was calculated as follows:

$$J_a = \frac{(A_r + R_d) \cdot (4.5 \cdot C_i + 10.5 \cdot \Gamma*)}{(C_i - \Gamma*)}$$

The intercellular CO_2 concentration is a critical variable in this determination. Were the stomatal aperture not uniform across the leaf surface, the value for J_a obtained from gas exchange data would lead to erroneous results (Downton et al. 1988). In order to verify this, the apparent electron transport rate (ETR) was estimated from fluorescence yield data as:

$$ETR = Yield \cdot PPFD \cdot 0.81 \cdot 0.5$$

The factor 0.81 is the coefficient of light absorption measured on adult leaves of Cabernet Sauvignon. The factor 0.5 was used to obtain the rate of O_2 evolution as described by Ögren and Evans (1993). Taking into account all data (days A, B, C and D), a positive correlation ($r = 0.684$, $p<0.001$) was found between J_a and ETR. This suggests a uniform distribution of stomatal apertures across the leaf and therefore a reasonably accurate estimation of J_a from gas exchange data.

Results

Light climate and leaf position (Experiment 1)

Data on day A were collected under conditions of intermittent cloudiness. The PPFD available for leaves at the various nodes along the shoot and thus varying in age differed significantly during the day (data not presented). Daily averages of assimilation rate and PPFD were always higher for sun- than shade-adapted leaves irrespective of leaf position (Figure 1). In relation to PPFD there was a steady decrease of A_r along the shoot for sun-leaves and there was a strong

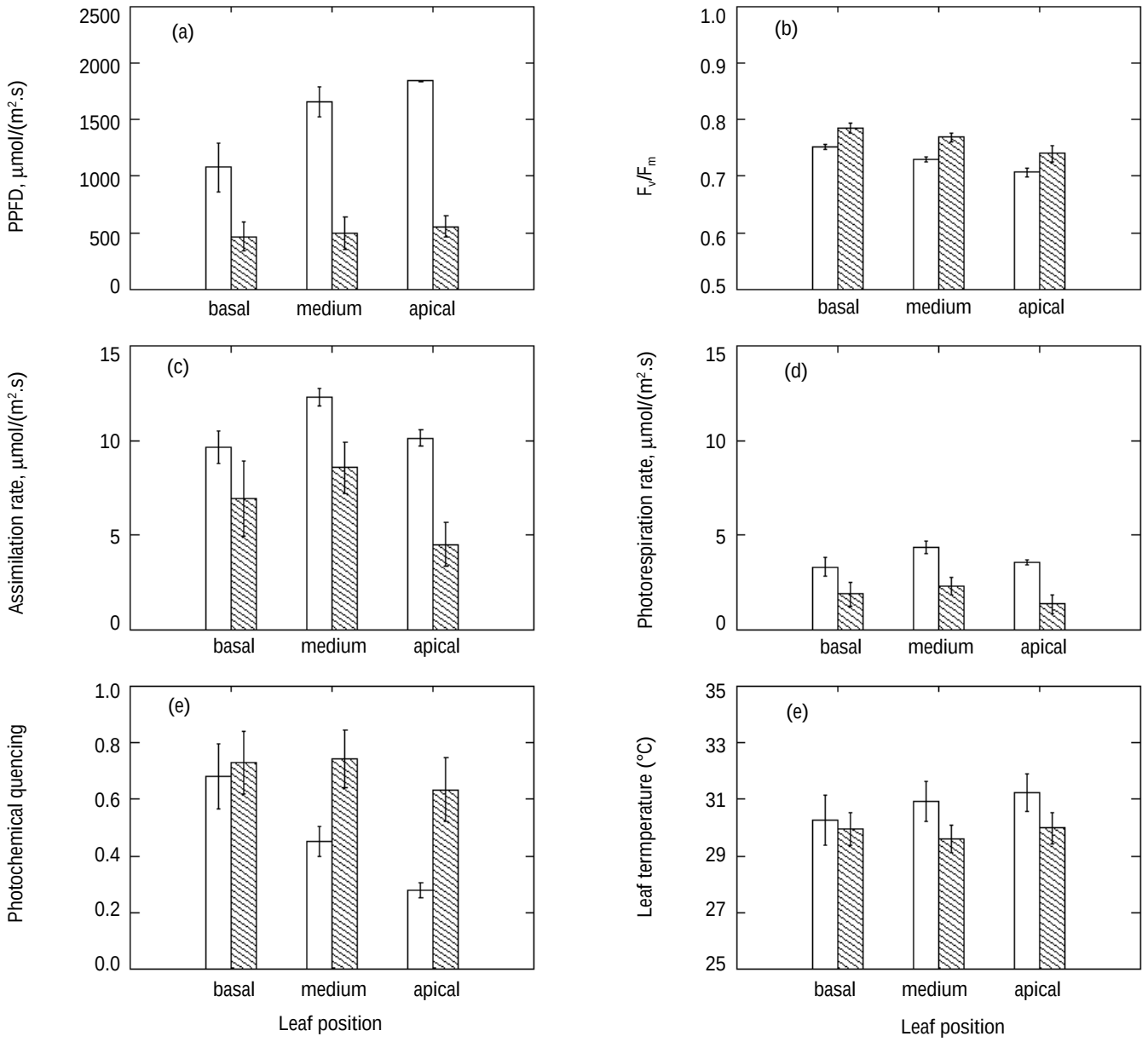

Figure 1. Measurements of light-related processes in leaves of Cabernet Sauvignon adapted to sun (□) and shade (▨)and in basal, medium and apical shoot positions. Data, collected on 1 December at 09.00, are shown for (a) PPFD, (b) F_v/F_m ratio, (c) assimilation rate (A_r), (d) photorespiration rate (PH), (e) photochemical quenching (q_P) and (f) leaf temperature. n = 16.

decrease for apical shade-leaves with a somewhat higher rate for medium shade leaves (Figures 1a, 1c). Photorespiration and leaf temperature of shade- and sun-adapted leaves were similar with regard to their leaf position, but were always lower in shade- than sun-adapted leaves irrespective of leaf position (Figures 1d, 1f). Maximum quantum yield of PSII photochemistry (F_v/F_m) and photochemical quenching of sun-adapted, and to a lesser extent of shade-adapted leaves, were inversely related to their PPFD (Figures 1b, 1e). This suggests that young apical leaves had more closed PSII centres ($1–q_P$) associated with a lower CO_2 assimilation rate. This result was confirmed by the photoinhibition treatment in the laboratory at 500 and 1500 µmol/(m².s) (Figure 2a). After 15 min of dark relaxation, following the exposure to the photoinhibition treatment, q_N began to decline in a linear fashion, a pattern similar to that observed by Ögren and Rosenqvist (1992). Under the high light of 1500 µmol/(m².s) the rate of q_N relaxation of apical leaves slowed relative to that of medium leaves. Therefore, q_I (typically assessed after 15 min of dark-relaxation) and the percentage of F_v/F_m inhibition were significantly higher when young apical, as compared to mature medium leaves, were treated with high light (Figures 2 b, 2c).

Photorespiration and photoinhibition of photosynthesis under different light conditions (Experiments 1 and 2)

Variable sunight with intermittent clouds

On day A, with variable light and intermittent cloud, A_r, PH, J_a and stomatal conductance of shade- and sun-adapted leaves were highest in the morning and progressively declined throughout the day (09.00, Table 1). The ratio of F_v/F_m of sun- and shade-adapted leaves was lower in the morning than later in the day. Generally, A_r, PH and J_a were positively related to PPFD, and the F_v/F_m ratio was negatively related to it.

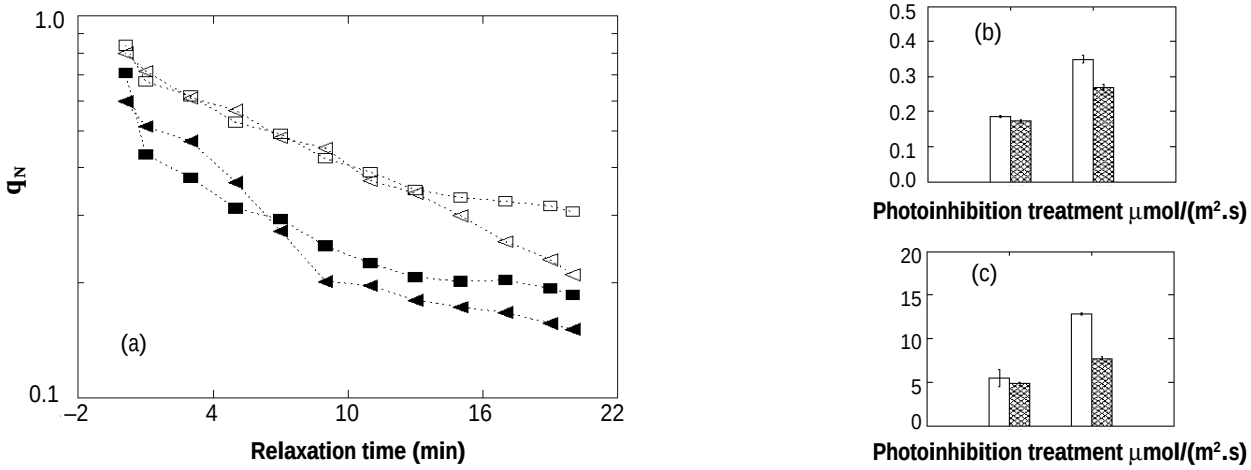

Figure 2. Measurements of photoinhibition on leaf discs. The discs, obtained from medium-placed adult and apical immature leaves of Cabernet Sauvignon, were exposed to high and low light intensities (1500 μmol/(m².s) and 500 μmol/(m².s) respectively), and measurements were made in the dark after 15 min of dark relaxation subsequent to exposure. Values represent the averages of sun- and shade-adapted leaves. **(a)** Semi-logarithmic plot of rate of relaxation of q_N recorded every 2 min for 20 min after exposure of apical leaves to 1500 (□) or 500 (■) μmol/(m².s) light or exposing medium leaves to 1500 (◁)or 500 (◀) μmol/(m2.s) light; **(b)** the relaxation of qi; **(c)** per cent of F_v/F_m inhibition. In (b) and (c) the medium leaves ⊠and apical leaves □were assessed after 15 min dark relaxation. Vertical bars in (b) and (c) represent standard errors. n = 6.

The relative difference between the F_v/F_m ratios of the two types of leaves remained virtually unchanged throughout the day.

On day B, a cloudy day (Table 2), diurnal variability was less evident than on day A because of the more uniform light conditions. At 09.00, when the difference of PPFD between shade- and sun-adapted leaves was largest, F_v/F_m of sun-adapted leaves was 10% lower than F_v/F_m of shade-adapted leaves. High A_r, PH and J_a always coincided with high PPFD. Leaf water potential never declined below –1.3 MPa and stomatal conductance remained above 100 mmol/(m².s) throughout, indicating that plants did not suffer from water stress.

Full sunlight

On sunny days (days C and D) the differences between sun- and shade-adaptation were more evident. On day C, a rise in the PPFD of sun-adapted leaves from 1500 to 1800 μmol/(m².s) between 09.00 and 13.00 was not followed by a concomitant increase in A_r but in PH (Table 3). In the morning A_r was 34% lower in shade- than in sun-adapted leaves but this difference had decreased to 8% by midday. Differences in PH and J_a between light treatments also decreased from morning to midday but were still considerable (40% and 29% respectively). Differences in the ratio of F_v/F_m between shade- and sun-adapted leaves had decreased from 2.5% in the morning to 37% by midday and this was

Table 1. Measurements on day A (1 December) on adult shade- and sun-adapted leaves in medium position, experiment 2. Mean values of assimilation rate, photorespiration rate, electron transport rate, F_v/F_m, intercellular level of CO_2, stomatal conductance and PPFD are shown together with values for per cent change between the two types of leaves (on the basis of sun leaves). Observations (n = 48) made at 09.00, 13.00 and 17.00. The standard error (SE) of the population mean is given. For details of variables see 'Materials and methods'.

	Leaf type	09.00		13.00		17.00		SE
		Mean	%change	Mean	%change	Mean	%change	
Assimilation rate, μmol/(m².s)	sun	10.9	27.5	7.0	32.9	4.8	43.9	0.4
	shade	7.9		4.7		2.7		
Photorespiration rate, μmol/(m².s)	sun	3.8	44.7	3.6	30.6	1.8	47.2	0.2
	shade	2.1		2.5		0.9		
Electron transport rate, μmol/(m².s)	sun	108.3	36.7	86.9	47.1	50.9	45.2	5.4
	shade	68.6		46.0		27.9		
F_v/F_m	sun	0.741	4.9	0.753	5.2	0.751	4.9	0.004
	shade	0.777		0.792		0.788		
Intercellular CO_2, ppm	sun	210.8	21.4	211.8	17.3	240.7	6.0	4.0
	shade	255.9		248.5		255.1		
Stomatal conductance, mmol/(m².s)	sun	171	16.4	118	5.9	100	29	8
	shade	199		111		72		
Photon flux density, PPFD, μmol/(m².s)	sun	1400	64	700	57	600	50	66
	shade	500		300		300		

Table 2. Measurements on day B (11 December) on adult shade- and sun-adapted leaves in medium position, experiment 2. Mean values of net assimilation rate, photorespiration rate, electron transport rate, F_v/F_m, intercellular level of CO_2, stomatal conductance, PPFD and leaf water potential are shown together with values for per cent change between the two types of leaves (on the basis of sun leaves). Observations (n = 48) made at 09.00, 13.00 and 17.00. The standard error (SE) of the population mean is given. For details of variables see 'Materials and methods'.

	Leaf type	09.00		13.00		17.00		SE
		Mean	%change	Mean	%change	Mean	%change	
Assimilation rate, µmol/(m².s)	sun	12.9	34.9	12.4	41.9	7.2	45.8	0.5
	shade	8.4		7.2		3.9		
Photorespiration rate, µmol/(m².s)	sun	4.6	47.8	5.1	51.0	2.5	48.0	0.2
	shade	2.4		2.5		1.3		
Electron transport rate, µmol/(m².s)	sun	129.9	40.5	132.0	44.8	69.9	43.5	5.5
	shade	77.3		72.8		39.5		
F_v/F_m	sun	0.723	10.0	0.812	1.8	0.810	0.7	0.09
	shade	0.795		0.827		0.816		
Intercellular CO_2, ppm	sun	219.2	11.3	222.7	16.3	249.0	9.8	3.3
	shade	243.9		258.9		273.5		
Stomatal conductance, mmol/(m².s)	sun	232	19.4	261	17.6	186	27.4	9.0
	shade	187		215		135		
PPFD, µmol/(m².s)	sun	1000	60.0	1000	50.0	400	50.0	55
	shade	400		500		200		
Leaf water potential, MPa	sun	–1.23	16.3	–1.30	15.4	–0.59	13.6	0.05
	shade	–1.03		–1.10		–0.51		

Table 3. Measurements on day C (5 January) on adult shade- and sun-adapted leaves in medium position, experiment 2. Mean values of net assimilation rate, photorespiration rate, electron transport rate, F_v/F_m, intercellular level of CO_2, stomatal conductance, PPFD and leaf water potential are shown together with values for per cent change between the two types of leaves (on the basis of sun leaves). Observations (n = 48) made at 09.00, 13.00 and 17.00. The standard error (SE) of the population mean is given. For details of variables see 'Materials and methods'.

	Leaf type	09.00		13.00		17.00		SE
		Mean	%change	Mean	%change	Mean	%change	
Assimilation rate, µmol/(m².s)	sun	12.6	34.1	12.5	8.0	8.3	38.6	0.4
	shade	8.3		11.5		5.1		
Photorespiration rate, µmol/(m².s)	sun	3.2	56.3	5.5	40.0	3.7	54.1	0.2
	shade	1.4		3.3		1.7		
Electron transport rate, µmol/(m².s)	sun	105.3	43.3	140.1	28.6	91.2	44.5	5.2
	shade	59.7		100.0		50.6		
F_v/F_m	sun	0.732	2.5	0.539	36.7	0.706	10.9	0.013
	shade	0.750		0.737		0.782		
Intercellular CO_2, ppm	sun	208.3	16.3	192.1	13.5	176.1	30.4	4.7
	shade	242.3		218.1		229.7		
Stomatal conductance, mmol/(m².s)	sun	167	15.0	152	9.9	87	6.9	7
	shade	142		167		81		
PPFD, µmol/(m².s)	sun	1500	73.3	1800	55.6	800	50.0	86
	shade	400		800		400		
Leaf water potential, MPa	sun	–0.79	20.3	–1.43	16.1	–0.83	38.6	0.05
	shade	–0.63		–1.20		–0.51		

most likely caused by photoinhibition, as proposed by Powles (1984). Midday values for water potential (–1.43 MPa) and stomatal conductance (152 mmol/(m².s) suggest that vines did not suffer from water stress.

On day D, the maximum PPFD of sun-adapted leaves was recorded at 09.00 and of shade-adapted leaves at 13.00 (Table 4). Under both conditions of light exposure, an increase in PPFD was followed by a

Table 4. Measurements on day D (12 February) on adult shade- and sun-adapted leaves in medium position, experiment 2. Mean values of net assimilation rate, photorespiration rate, electron transport rate, F_v/F_m, intercellular level of CO_2, stomatal conductance, PPFD and leaf water potential are shown together with values for per cent change between the two types of leaves (on the basis of sun leaves). Observations (n = 48) made at 09.00, 13.00 and 17.00. The standard error (SE) of the population mean is given. For details of variables see 'Materials and methods'.

	Leaf type	09.00		13.00		17.00		SE
		Mean	%change	Mean	%change	Mean	%change	
Assimilation rate, µmol/(m².s)	sun	10.6	34.9	11.1	17.1	8.9	43.8	0.4
	shade	6.9		9.2		5.0		
Photorespiration rate, µmol/(m².s)	sun	2.1	52.4	3.8	39.5	3.2	50.0	0.2
	shade	1.0		2.3		1.6		
Electron transport rate, µmol/(m².s)	sun	80.5	40.5	107.4	28.0	88.9	46.1	4.5
	shade	47.9		77.3		47.9		
F_v/F_m	sun	0.559	34.0	0.602	10.0	0.664	10.1	0.014
	shade	0.749		0.662		0.731		
Intercellular CO_2, ppm	sun	229.4	15.4	205.9	11.5	205.1	8.1	4.1
	shade	264.8		229.6		221.8		
Stomatal conductance, µmol/(m².s)	sun	164	10.4	151	4.6	118	37.3	7.0
	shade	147		158		74		
Photon flux density PPFD, µmol/(m².s)	sun	1500	80.0	1400	42.9	1000	60.0	77
	shade	300		800		400		
Leaf water potential, MPa	sun	–0.92	5.4	–1.37	14.6	–1.09	28.4	0.39
	shade	–0.87		–1.17		–0.78		

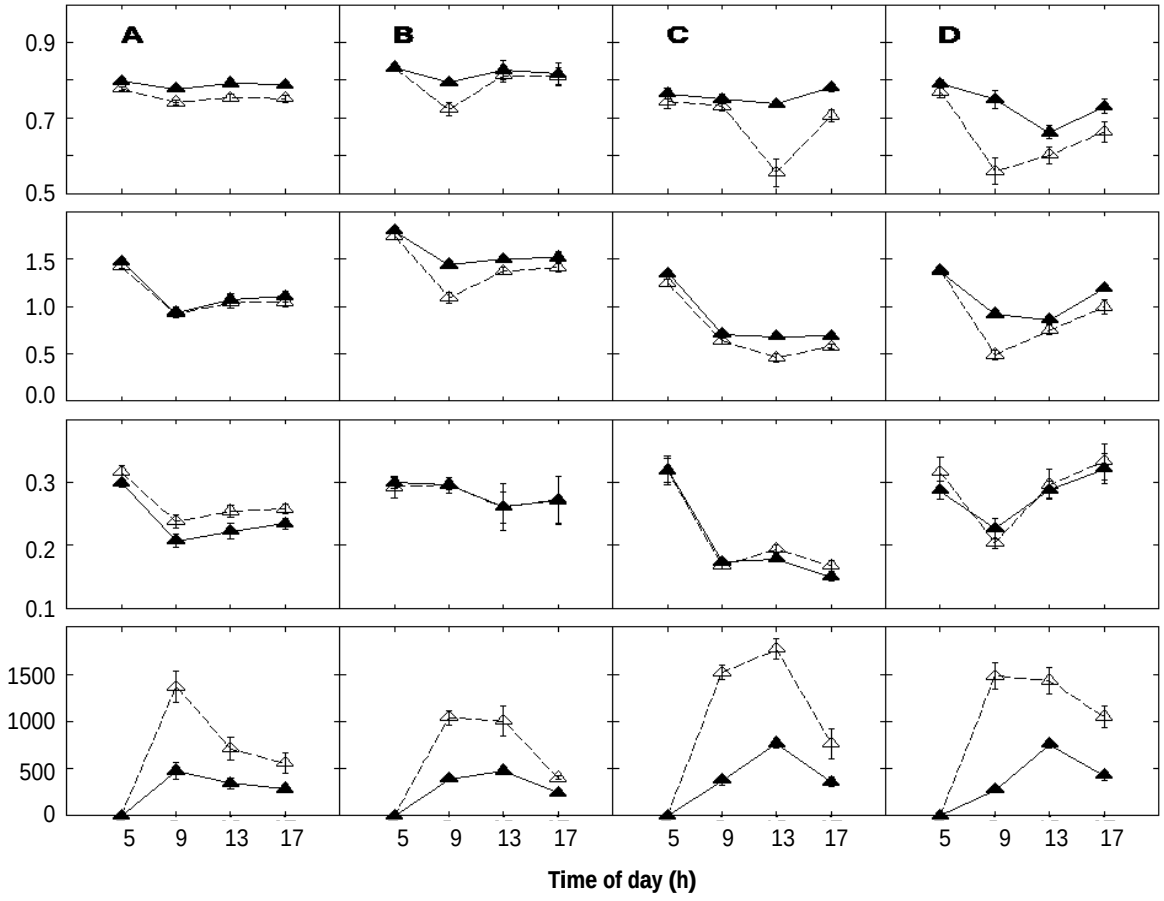

Figure 3. Diurnal course of light-dependent processes of sun-adapted (△) and shade-adapted (▲) leaves in the field. Measurements on Cabernet Sauvignon leaves were made of (a) F_v/F_m, (b) maximum (F_m), (c) minimum (F_0) fluorescence, and (d) PPFD on four days under intermittently cloudy (A, 1 December), continuously cloudy (B, 11 December) and clear (C, 5 January; D, 12 February) conditions. Vertical bars represent standard errors. n = 8.

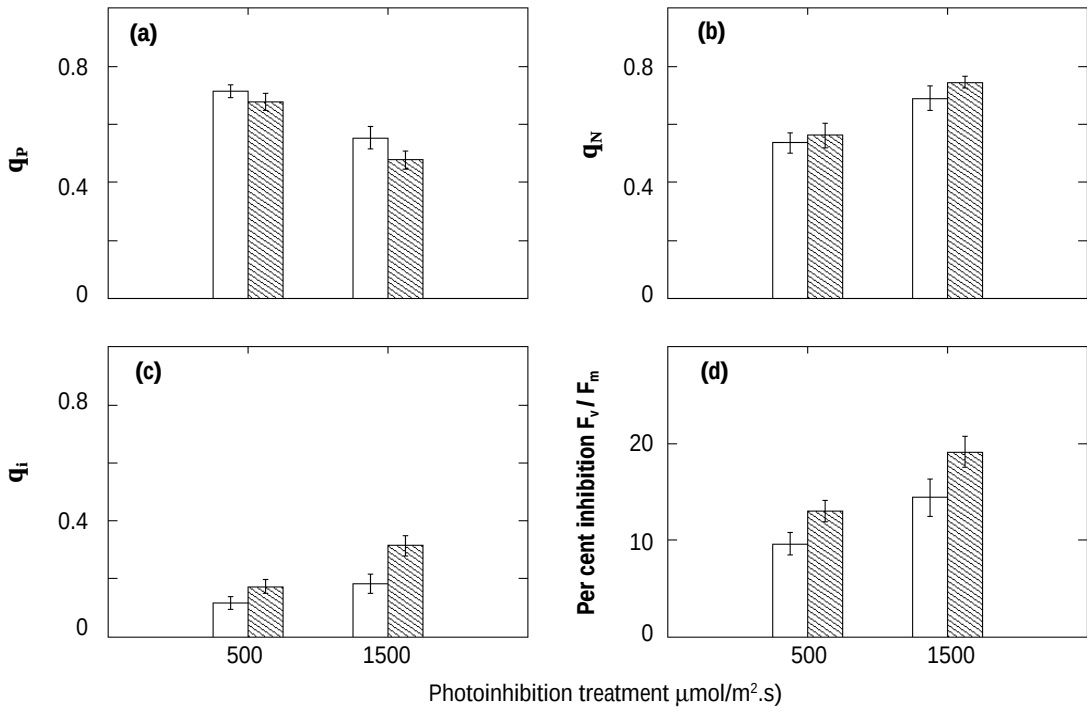

Figure 4: Measurements of quenching coefficients and photoinhibition on leaf discs The discs, from Cabernet Sauvignon leaves, were obtained from medium adult leaves adapted to sun (®) or shade (®) after exposure to high or low light. Measurements of q_P (a) and q_N (b) were obtained immediately following exposure to 1500 or 500 µmol/(m²·s) light. The relaxation of q_I (c) and percentage of F_v/F_m inhibition (d) were assessed after 15 min of dark relaxation. Vertical bars in a–d are standard errors. n = 31.

significant decrease of F_v/F_m and maximal light differences were followed by a correspondingly large change in F_v/F_m (34% at 09.00). Values of leaf water potential and stomatal conductance suggest that plants did not suffer from water stress.

Fluorescence components

Analysis of the components of the F_v/F_m ratio indicates that photoinhibition occurred. The ratio of F_v/F_m was always lower in sun- than in shade-adapted leaves (Figure 3). Particularly on clear days (days C and D), when PPFD was at its maximum, both shade- and sun-adapted leaves showed reduced photochemical efficiency (F_v/F_m). The decline of F_v/F_m was less pronounced however in shade-adapted leaves, probably because they received a lower PPFD than sun-adapted leaves. Maximum fluorescence (F_m) always decreased in concert with F_v/F_m, but F_0 did not match the expected deactivation pattern. Bolhar-Nordenkampf et al. (1991) made a similar observation. The ratio of F_v/F_m declined at PPFD levels ranging from 500–1200 µmol/(m².s) (Figure 3, day B), indicating that even relatively low light levels may cause photoinhibition. Rosenqvist et al. (1991) reported a similar observation for willow trees (*Salix)*. On day D, a clear day, shade- and sun-adapted leaves received peak PPFD levels at different times of the day. The observed PPFD levels were inversely related to the corresponding measurements of F_m and F_v/F_m but not to those of F_0. On both sunny days C and D, F_m and F_v/F_m had recovered by late afternoon (17.00), thereby almost completely reversing photoinhibitory effects.

Study of q_N-relaxation in shade- and sun-adapted leaves

After dark adaptation, discs from shade-and sun-adapted leaves were treated for 30 min with high and low light of 500 and 1500 µmol/(m².s). Only at the higher light level was q_P of shade-adapted leaves significantly lower than that of sun-adapted leaves (Figure 4a). Although q_N was consistently higher in shade- than sun-adapted leaves, the differences were not significant at either light level. The percentage of F_v/F_m inhibition and q_i (Figure 4c, 4d) were significantly higher for discs from shade- compared to sun-adapted leaves, irrespective of the light level. Overall, a positive correlation was found between q_i and both q_N and the percentage of F_v/F_m inhibition, and a negative correlation between q_i and q_P (data not presented).

Discussion

Diurnal observations

The diurnal course of A_r, PH, stomatal conductance and J_a generally showed the expected positive relationship with solar radiation (PPFD), but showed an inverse relationship with F_v/F_m and water potential. Consequently, in shade-adapted leaves A_r was always lower than in sun-adapted leaves. On sunny days, A_r of sun-adapted leaves began to decline (day C) or rose only slowly (day D) between 09.00 and when solar radiation reached its highest levels at 13.00 whereas, in contrast, A_r of shade-adapted leaves continued to increase strongly. Consequently, at 13.00 on sunny days, both shade- and sun-adapted leaves attained similar levels of A_r. The trend towards saturation of A_r under high light was partly attributable to an increase

in PH. Therefore, at light intensities at which A_r was becoming saturated, photorespiration constituted a mechanism by which a large proportion of the potential CO_2 assimilation rate, 5.5 of 18 µmol/(m^2.s) was dissipated as excess energy, thereby protecting PSII from the detrimental effects of high radiation (day C, 13.00; Table 3).

On the other hand, photorespiration increased at about the same rate in sun- and shade adapted leaves, and therefore did not fully account for the decreasing differences in A_r between light treatments from 09.00 to 13.00. Moreover, saturation of A_r under high light always coincided with a steep decrease in the ratio of F_v/F_m, providing strong evidence that leaves were experiencing photoinhibition. Although midday (13.00) water potential was slightly lower in sun- than in shade-adapted leaves, stomatal conductance during the time span from 09.00 to 13.00 was similar between the light treatments which suggests that there were no stomatal limitations.

Analysis of the fluorescence components (Figure 3) showed that reductions in F_v/F_m (Fig. 3a), coinciding with high PPFD (Fig. 3d), were mainly caused by a decline in F_m (Fig. 3b), while the behaviour of F_0 (Fig. 3c) was unclear. We saw an increase in F_0 on day C, 13.00, but failed to see it regularly. For instance, F_0 remained unchanged or even decreased in response to high light on days B and D. Normally, a decline in F_m which follows exposure to high light, is paralleled by an increase in F_0. Bolhar-Nordenkampf (1991) observed a similar erratic behaviour of F_0 in response to high light, and gave as possible causes measuring errors in the determination of F_0 and F_m or biological variability. At relatively low F_v/F_m ratios, even small changes in F_0 or F_m would result in considerable changes in the F_v/F_m ratio. Alternatively, different stages of photoinhibitory damage may occur in various patches on the leaf lamina (Critchley 1988). On days B (09.00) and D (09.00), when F_0 values were contradictory to those of F_m, such damage may have occurred.

The results of the diurnal observations showed that photoinhibition of photosynthesis was consistently present at high light, typically above 1500 µmol/(m^2.s). It probably represents a mechanism of protecting PSII from excessive light. On the other hand, we also observed photoinhibition at relatively low light intensities of around 1000 µmol/(m^2.s) (day B; Table 2) which, according to Düring (1988), is just below the level of light saturation for grapevines. Photoinhibition therefore occurred in full sun and at sub-optimal light levels in shade-and sun-adapted leaves but was always completely reversible.

Taking into account all data, A_r and F_v/F_m closely followed PPFD at a given leaf position. Therefore, under uniform light conditions, i.e. on sunny or overcast days, the instantaneous PPFD values may be sufficient to predict photoinhibition of photosynthesis instead of using values of the absorbed energy over a given time period as suggested by Ögren and Sjöström (1990).

Photoinhibition treatment and quenching analysis

Subjecting shade- and sun-adapted leaves to a photoinhibitory treatment in the laboratory showed that adaptation to low light regimes sensitised leaves to photoinhibition. Shade- as compared to sun-adapted leaves were always more strongly photoinhibited, as expressed by the percentage of F_v/F_m inhibition after exposure to high and low light in the laboratory (respectively 1500 and 500 µmol/(m^2s); Figure 4). The percentage of F_v/F_m inhibition represents the percentage reduction of the F_v/F_m ratio after applying the photoinhibitory treatment. Furthermore, shade-adapted leaves showed a larger relative increase in inhibition from the low to the high light treatment than sun-adapted leaves.

Quenching analysis using modulated chlorophyll fluorescence strongly supported this result, showing a close positive relationship between the percentage of F_v/F_m inhibition and non-photochemical photoinhibitory quenching (q_I). Modulated chlorophyll fluorescence allows the measurements of coefficients related to photochemical (q_P) and non-photochemical (q_N) components of fluorescence which reflect different levels of the photosynthetic process.

Photochemical quenching (q_P) is determined by primary processes of photochemistry so that q_P decreases, with increasing fluorescence, in proportion to the closure of reaction centres (electron acceptors) in PSII. Non-photochemical quenching (q_N) primarily represents an increase in non-radiative dissipation of light energy through heat and is related to processes in the thylakoid membrane and to carbon metabolism. The coefficient of q_I may be considered as that part of q_N that is due to photoinhibition (Schreiber et al. 1994). Non-photochemical quenching normally relaxes after a period of dark adaptation (Schreiber et al. 1994). Therefore, q_I is attributible to photoinhibition, being the part of q_P induced by high light that still persists after normal relaxation, in our case after 15 min. We recorded progressively higher q_I values for shade- compared to sun-adapted leaves following a high (1500 µmol/m^2.s) but not a low intensity (500 µmol/m^2.s) photoinhibitory treatment (Figure 4c). The response of q_I was comparable to that of the percentage inhibition of F_v/F_m (Figure 4d), again highlighting the more severe photoinhibition of shade- than of sun-adapted leaves under high light. Conversely, high light decreased q_P more severely in shade- than sun-adapted leaves, whereas low light caused a less pronounced reduction. There was an inverse relationship between q_I and q_P. A decrease in the q_P values is indicative of an increasing proportion of closed PSII reaction centres ($1–q_P$) and hence a 'downregulation' of photosynthesis (Horton and Hague 1988). Ögren and Rosenqvist (1992) obtained a similar relationship with a range of plant species, showing that these mechanisms are common in the plant kingdom.

A practical consideration from the above result is that previously shade-adapted leaves inside a vine canopy are likely to suffer from photoinhibition when exposed to full sun as a consequence of leaf removal or summer pruning. The degree of inhibition experienced by newly exposed leaves would be progressively more severe with increasing light levels.

In summary, the above results indicate that in *Vitis vinifera* photorespiration and photoinhibition of photosynthesis appear to be important means of protecting PSII from excessive energy excitation. This seems particularly important at high levels of solar radiation, but photoinhibition also occurs well below the maximum levels of PPFD, thereby decreasing the maximal quantum yield of the PSII photochemistry.

Effects of leaf position and age

Leaves on basal nodes received less light than leaves in medium position on the shoot when both were exposed to the sun, and correspondingly the basal leaves had lower rates of net photosynthesis and photorespiration. The light gradient resulting from differences in leaf position was more pronounced in sun- than shade-adapted leaves, and a large proportion of the differences in A_r between leaf positions of sun-adapted leaves were due to this gradient. However, A_r of shade- and sun-adapted leaves was affected also by leaf age. Young apical leaves in the sun, which received the highest level of PPFD of all leaf categories, had a much lower A_r than medium leaves in the sun whose PPFD was slightly lower (Figure 1). Similarly, a significant reduction in A_r from apical (immature) to medium (mature) leaves was apparent on shade-adapted shoots, although leaves in either position received similar levels of PPFD.

It is well documented that immature vine leaves have a significantly lower A_r than fully mature leaves under a given light regime (Kriedemann et al. 1970, Downton and Grant 1992). Our data suggest that in sun-adapted apical leaves the observed reduction in A_r may be partly attributable to photoinhibition. Firstly, the difference in the percentages of photorespiration, 40% in sun-adapted apical and 33% in sun-adapted medium leaves, could not fully account for the observed differences in A_r; the remaining portion was probably due to photoinhibition. Secondly, exposing the dark-adapted leaf discs to light of high intensity, 1500 µmol/(m^2.s), confirmed that immature, apical leaves were more susceptible to photoinhibition than mature leaves because discs from immature leaves had a much higher q_I and percentage of F_v/F_m inhibition (Figures 2b, 2c). As pointed out earlier, the coefficient of q_I represents that part of non-photochemical quenching (q_N) that is due to photoinhibition (Schreiber et al. 1994). Also, the much lower values for q_P in apical sun-adapted leaves than in shade-adapted apical leaves indicated a higher proportion of closed reaction centres under high light. Possibly, immature leaves in the sun are more susceptible to photoinhibition because they are short in Calvin-cycle enzymes and therefore lack a fully developed metabolism to provide for energy dissipation through A_r and photorespiration. In addition to the depressing effect of a rising PPFD on q_P between basal and apical leaves on sun-adapted shoots, there also appeared to be an interactive effect between leaf age and position, leading to a further reduction of q_P with decreasing leaf age (Figure 1). Leaf position on sun-adapted shoots is characterised by an increasing light level with decreasing leaf age towards the shoot tip, leading to interactive effects between leaf age and light level. Thereby the effects of photoinhibition seemed to be exacerbated.

Practical implications

The processes of photoinhibition and photorespiration are known to depress the photosynthetic performance of leaves and are enhanced by high levels of solar radiation, high temperature and water stress (Powles 1984, Ludlow and Powles 1988). Such conditions are often encountered in Australian vineyards and therefore it is important to assess the impact of photoinhibition and photorespiration on vine performance in the field.

In our experiments, photoinhibition occurred at levels of solar radiation, expressed as PPFD, that would be experienced by vines on an almost daily basis during the growing season. Although all leaf categories showed photoinhibition, immature and shade-adapted leaves were more sensitive to it than mature and sun-adapted leaves. Such a response may have important practical consequences with respect to the choice of systems of trellising and canopy management. For example, measures such as leaf removal and topping may result in a sudden and permanent exposure of previously shaded leaves to full sunlight. In addition to the removal of fully functional leaves, this may lead to significant photoinhibitory effects and possibly to loss of production. The proportion of shade *v.* sun leaves may vary depending on the chosen trellising system, puning level and shoot manipulation. Normally, even shaded leaves experience transient exposure to sunflecks during the course of the day and thus contribute to the total assimilation within the foliage canopy (Kriedemann et al. 1973). However, many such shade-adapted leaves may suffer from increased sensitivity to photoinhibition, and therefore may be impaired in their capacity to assimilate during and after transient exposure to full sunlight.. Thereby, their already limited contribution to overall photosynthetic production will be reduced even further.

Moreover, in a dense canopy with a high proportion of shaded and senescing leaves, the contribution to photosynthesis during ripening would mainly rely on relatively young exterior leaves which are more likely to suffer from photoinhibition. Therefore, it is desirable under growing conditions with continuously high light levels, as in the inland viticultural regions of Australia, to maintain an open canopy, characterised by a large leaf surface area yet good sub-penetration to interior leaf layers. Outside leaves then act as a shield but still allow sufficient light to penetrate to the interior and thus ensure a high photosynthetic efficiency of the foliage canopy.

We did not explore the interaction of photoinhibition with other stress conditions such as water-, salt - and high-emperature stress. Results from other sources suggest however that photoinhibition effects are exacerbated by these stresses and therefore are likely to lead to a significant reduction in yield and quality (Gamon and Pearcy 1990). The relatively recent development of modulated chlorophyll fluores-

cence provides a means for assessing the impact of environmental stress on the *in vivo* performance of grapevine leaves in the field. It could be used to screen new winegrape varieties and rootstocks for differences in stress tolerance and photosynthetic performance. Future work should evaluate the application of fluorescence assays for the rapid field screening of nursery stock. *In vivo* chlorophyll fluorescence techniques should also be tested for assessing the impact of environmental stresses on vine performance in the field. The study here presented would then serve to provide base data for such investigations of direct application to viticulture.

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