Light utilisation and thermal dissipation in light- and shade-adapted leaves of *Vitis* genotypes

T. Ortoidze[1]) and H. Düring[2])

[1]) Institute of Horticulture, Viticulture and Wine Production, Tbilisi, Georgia
[2]) Institut für Rebenzüchtung Geilweilerhof der Bundesanstalt für Züchtungsforschung an Kulturpflanzen, Siebeldingen, Deutschland

Summary

Light responses of chlorophyll fluorescence and photosynthetic parameters were determined using light- and shade-adapted leaves of cvs Müller-Thurgau, Riesling and Dornfelder. The quantum yield of photochemical energy conversion (Y) decreased with increasing light intensity (PAR), shade-adapted leaves showing a stronger decline already at very moderate PAR. For both leaf types the electron transport rate (ETR) increased with PAR but to distinctly higher values in light-adapted leaves. Correspondingly, rates of maximum CO_2 assimilation at ambient CO_2 concentration (A_{max}) and stomatal conductance at A_{max} were higher in light-adapted than in shade-adapted leaves, cv. Dornfelder always showing higher photosynthetic performance than cvs Riesling and Müller-Thurgau. Maximum ETR of light-adapted leaves of 11 genotypes varied between 85 (Müller-Thurgau) and 220 (Lemberger), red genotypes generally showing higher values than white genotypes. In light-adapted leaves of cvs Müller-Thurgau, Riesling and Dornfelder non-photochemical quenching (NPQ) increased up to PAR = 2,350 µmol m^{-2} s^{-1}. In shade-adapted leaves of Müller-Thurgau and Riesling NPQ reached saturation already at PAR = 500 µmol m^{-2} s^{-1}, indicating a limited capacity for thermal dissipation of energy; in contrast, in shade-adapted leaves of Dornfelder the NPQ increase was similar to that of light-adapted leaves.

The ß-carotene and lutein content and the xanthophyll pool size (violaxanthin + antheraxanthin + zeaxanthin) of light-adapted leaves (cvs Müller-Thurgau, Riesling and Dornfelder) were higher than in shade-adapted leaves. In light-adapted leaves the xanthophyll pool was lowest for Müller-Thurgau and highest for Dornfelder indicating a higher capacity of thermal dissipation of energy for the latter variety. It is concluded that the high photosynthetic light use and photoprotective ability of Dornfelder contribute to its lower proportion of excess PAR and reflect its superior ability for adaptation to high light.

K e y   w o r d s : light utilisation, energy dissipation, adaptation to light and shade, photosynthesis, chlorophyll fluorescence, xanthophyll pool.

Introduction

Leaves of field-grown grapevines are exposed to most variable changes of light, namely to its intensity and speed. E.g., diurnal changes of light intensity at the outer part of canopies often range from zero (night) to approximately 2000 µmol m^{-2} s^{-1} (noon) while inside of canopies leaves are generally exposed to much more reduced light intensities. Moreover, incident light intensity may change within minutes, e.g. due to clouds, or seconds, due to sun flecks (PEARCY 1990). Light use in photosynthesis is significantly different between light- and shade-adapted leaves, both trying to find 'a path between maximising light interception for photosynthesis and minimising the potential for damage arising from the over-excitation of the photosynthetic apparatus' (LONG *et al.* 1994). While in light-adapted leaves at lower light intensity (100-200 µmol m^{-2} s^{-1}) the absorbed light is utilised almost entirely by photosynthesis, at full sun light the utilisation drops to approximately 10 %; the decline of light utilisation at high light can even be stronger if other ambient factors, e.g. non-optimal temperatures or low plant water status leading to stomatal closure, are limiting rates of CO_2 assimilation (JONES 1983, LONG *et al.* 1994). According to its intensity and duration excess light can cause transient or permanent damage of the photosystem II (PSII), the most vulnerable part of the photosynthetic apparatus with respect to light-induced damage (DEMMIG-ADAMS and ADAMS 1992, MAXWELL and JOHNSON 2000). There is much evidence that grapevines, as many other species, are able to protect PSII under high light conditions either by avoiding over-excitation of PSII by lowering energy absorption or by inactivation and repair cycles (reviews: TREBST 1994, MELIS 1999). Absorbed excess excitation energy can be dissipated by non-radiative (thermal) energy dissipation and, closely related herewith, the xanthophyll cycle (reviews: BJÖRKMAN and DEMMIG-ADAMS 1994, DEMMIG-ADAMS and ADAMS 1996, HORTON *et al.* 1996; grapevine: DÜRING 1999).

The aim of the present paper was to compare field-grown, light- and shade-adapted leaves of several grapevine genotypes with respect to their ability to adapt to high light by determining photosynthetic light use, rates of excessive light and photoprotective processes.

Material and Methods

In June 2000 fully expanded but not senescent leaves of 8-year-old field-grown Müller-Thurgau, Riesling and Dornfelder (Helfensteiner x Heroldrebe) vines grafted to Kober 5 BB as well as 8 other *Vitis* genotypes (see Tab. 1) of the experimental field of the Institute for Grapevine Breeding Geilweilerhof were cut at the proximal end of their petioles,

inserted into a water-containing vessel and cut again under water to avoid cavitation. Between 10.00 and 14.00 h (local time) light-adapted leaves were collected on cloudless days at 1,300-1,500 μmol quanta m^{-2} s^{-1} from sun-exposed upper parts of the canopy (vertical espalier), while shade-adapted leaves were collected within the same time span from the inner part of the canopy at 80-120 μmol quanta m^{-2} s^{-1}. Leaves were kept in dark during the brief transport to the laboratory.

Chlorophyll fluorescence of leaves was determined in the laboratory using a MINI-PAM Fluorescence Meter based on the modulation principle and a WinControl Software (Walz, Effeltrich, Germany). Leaves with their petioles in water were kept in darkness, either for 10-15 min or overnight (NPQ determination) and then inserted into a leaf-clip holder to determine light responses. Chlorophyll fluorescence parameters were calculated according to Schreiber et al. (1994) and Schreiber (1997):

Maximum quantum yield of PSII (Y):
$$F_m - F_o / F_m \qquad (1)$$
Effective quantum yield of PSII (Y):
$$F_m' - F / F_m' \qquad (2)$$
Relative electron transport rate (ETR):
$$Y \times 0.42 \times PAR \qquad (3)$$
Non-photochemical quenching (NPQ):
$$(F_m - F_m') / F_m' \qquad (4)$$

Light response curves were obtained from yield measurements at the end of 8 consecutive illumination periods (duration: 20 s, light intensity increasing from 0 to 2, 350 μmol m^{-2} s^{-1}).

Determination of 'excessive PAR' is explained in Fig. 1, line 'a' being the optimum line. This line starts at the origin, its ascent corresponds to the initially determined maximum quantum yield of PSII. E.g., at PAR = 500 μmol m^{-2} s^{-1} the second point of line 'a' is calculated as follows:
$$Y \times 0.42 \times 500 \text{ (Schreiber 1997).}$$
According to Schreiber et al. (1994) the deviation from the optimum quantum yield in the sense of 'excessive PAR' can be quantified by relating A - B to A' - B' (Fig. 1).

Maximum CO_2 assimilation (A_{max}) and stomatal conductance (g) at A_{max} were determined with field-grown leaves cut under water and exposed to light saturation (1,200 μmol m^{-2} s^{-1} for light-adapted and 400 μmol m^{-2} s^{-1} for shade-adapted leaves, Schott KL 1500 lamp; 25 $\pm$2 °C). Measurements were performed using a Walz-Porometer at ambient CO_2 concentration (350 $\pm$10 ppm). Gas exchange data were stored by a DES data recording unit (Walz, Effeltrich, Germany) and calculated according to von Caemmerer and Farquhar (1981).

For carotenoid analysis segments of light- and shade-adapted leaf blades from field-grown vines (diameter: about 25 mm) were exposed to high light (Schott KL 1500, 2,100 μmol m^{-2} s^{-1}) for 5 min to induce conversion of violaxanthin to antheraxanthin and zeaxanthin (Demmig-Adams and Adams 1996, Düring 1999). These segments were frozen immediately by liquid N_2 and stored at -23 °C.

Carotenoid analysis followed the procedure described by Thayer and Björkman (1990), for details see Düring (1999). Carotenoids of the xanthophyll cycle as well as ß-carotene
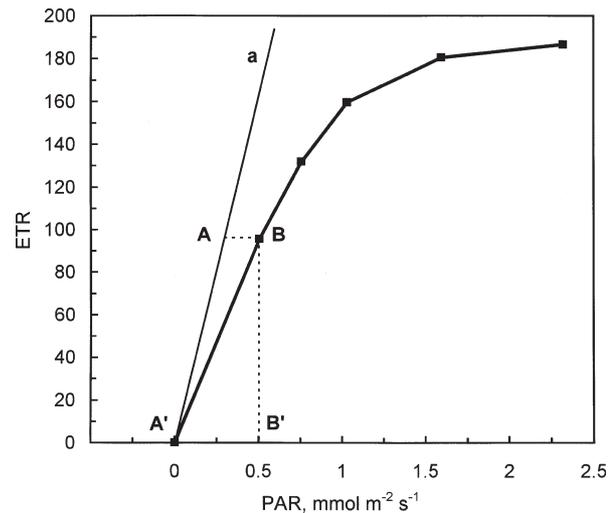

Fig. 1: Actual relative electron transport rate (ETR) as a function of light intensity (PAR) in comparison to the optimum quantum yield of a 'model leaf' (line a). Excessive PAR is determined by relating A-B to A'-B'. For details: Material and Methods.

and lutein were related to neoxanthin which was shown to be rather unaffected by light treatments (Björkman and Demmig-Adams 1994, Adams et al. 1999, Düring 1999).

Leaf diameter (thickness) was determined together with leaf sampling in the field using a micrometer-screw-system (resolution: 10 μm).

Results and Discussion

Light responses of photochemical processes: Measurements were started with dark-adapted leaves at the minimal level of fluorescence, F_o. Saturating light flashes led to a rapid increase of fluorescence to F_m, the maximum fluorescence in the dark-adapted state. Thereafter actinic light periods of increasing intensity were applied and, at the end of each, saturating light flashes. From the latter the maximum florescence in the light (F_m') is derived. After flashes, removal of actinic light allowed determination of F_o'. The efficiency of PSII photochemistry or quantum yield of PSII measures the proportion of light absorbed by chlorophyll associated with PSII that is used in photochemistry.

In an initial trial light response curves were obtained from field-grown light- and shade-adapted leaves, cvs Müller-Thurgau, Riesling and Dornfelder (Fig. 2). For all three varieties the quantum yield of shade-adapted leaves showed a stronger decline compared to light-adapted leaves which started already at very moderate PAR (500 μmol m^{-2} s^{-1}).

The relative electron transport rate (ETR) increased with light intensity in light- and shade-adapted leaves, maximum values of light-adapted leaves being distinctly higher than those of shade-adapted leaves. Since the maximum ETR values of light-adapted leaves of the three varieties differed significantly (Fig. 2) the experiments were extended to 8 other field-grown red and white genotypes. Tab. 1 indicates large genotypical differences, ETR values of light-adapted leaves ranging from 85 (cv. Müller-Thurgau) to 220 (cv. Lemberger). On the basis of these studies, ETR maxima were higher in
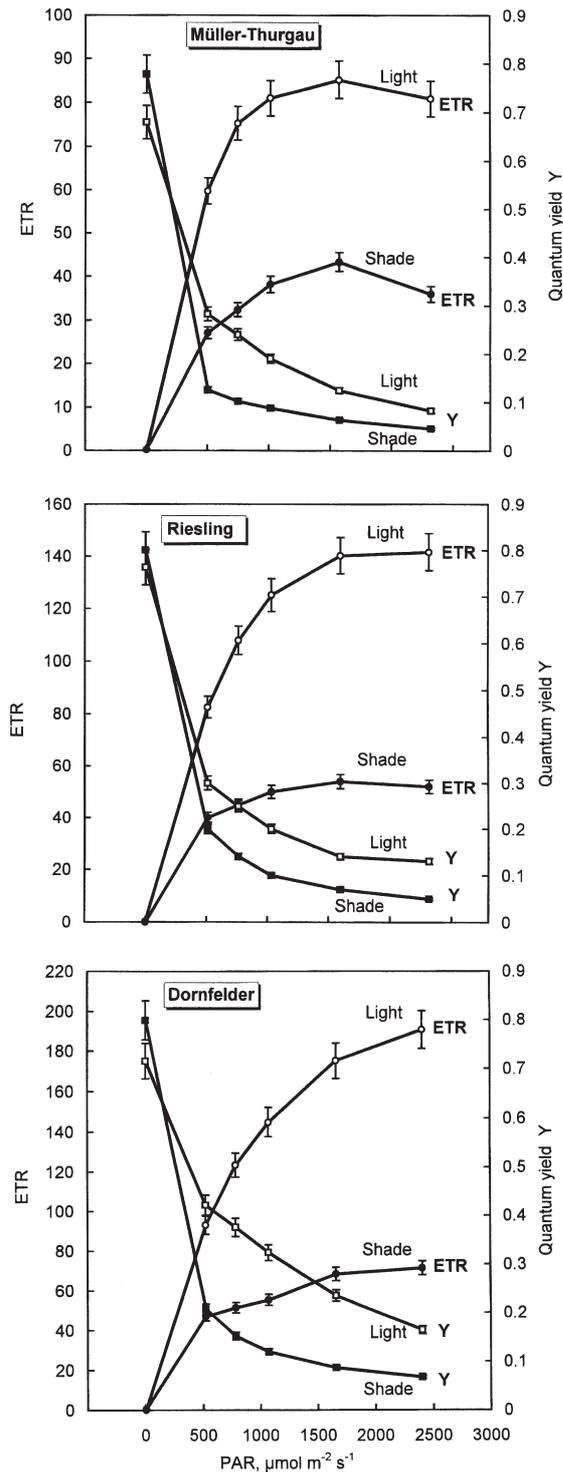
Fig. 2: Light response curves of the relative electron transport rate (ETR) and of quantum yield of light- and shade-adapted leaves of field-grown Müller-Thurgau, Riesling and Dornfelder leaves. Mean values of three independent experiments, bars denote confidence limits at the 5 % level.

Table 1

The maximum relative electron transport rate (ETR_{max}) obtained from light response curves of field-grown leaves of white (W) and red (R) *Vitis* genotypes. Mean values of 6 independent experiments. (For details: Fig. 2).

Genotype	ETR_{max}	conf.lim.[*)]
Müller-Thurgau. W	85.2	7.2
Riesling. W	141.6	6.8
Villard blanc. W	164.0	5.0
Airen. W	169.4	6.6
Gf.Ga 47-42. W	175.6	8.0
Regent. R	183.6	8.1
Dornfelder. R	190.6	8.7
V. amurensis. R	194.4	7.3
Heroldrebe. R	201.5	7.6
Helfensteiner. R	210.4	5.6
Lemberger. R	219.8	6.7

[*)] confidence limits at the 5 % level

According to GENTY *et al.* (1989) under constant laboratory conditions there is a strong linear relationship between ETR and the efficiency of carbon fixation. In fact, this appears to be substantiated by comparing the maximum CO_2 assimilation rates at ambient CO_2 partial pressure and saturating light conditions (A_{max}) obtained from light-adapted leaves of three varieties and the corresponding maximum ETR values (Fig. 2, Tabs 1 and 2).

Generally, light-adapted 'sun leaves' are reported to be thicker, to have a higher proportion of palisade cells which are longer than in shade-adapted leaves; these geometric properties of light-adapted leaves are ideal prerequisites for high light interception and use of photosynthetic CO_2 assimilation (NOBEL 1983, VOGELMANN 1993, ESCHRICH 1995). It can be assumed that in our experiments the higher photosynthetic performance of light-adapted leaves was associated with their higher leaf thickness (Tab. 2). Light-adapted leaves of Müller-Thurgau and Dornfelder differed significantly in both, leaf thickness and A_{max}.

Light responses of non-photochemical processes : It is generally accepted that in unstressed leaves after a long-term dark-adaptation (10-12 h) the efficiency by which energy is converted to heat is at its minimum and that changes in the non-photochemical quenching (NPQ) indicate changes of the efficiency of heat dissipation relative to the dark-adapted state (BILGER and BJÖRKMAN 1990, MAXWELL and JOHNSON 2000). Increases of NPQ with PAR can occur as a result of either protective processes against high light or of high light damage itself in the light-harvesting antenna of PSII (HORTON *et al.* 1996).

In field-grown light-adapted leaves of Müller-Thurgau, Riesling and Dornfelder NPQ increased with increasing light intensity and even at PAR >2,000 μmol m^{-2} s^{-1} no saturation of thermal dissipation was observed, *i.e.* the maximum capacity of thermal dissipation was not yet reached. In light-adapted leaves of Müller-Thurgau NPQ increased to higher values than in Riesling and Dornfelder. The latter two varie-

leaves of red varieties than of white varieties. However, more experiments will be necessary to corroborate these results. The higher ETR values in light-adapted leaves may be due to the higher number of photosynthetic electron transport chains per total chlorophyll found in sun-type chloroplasts (LICHTENTHALER 1996).

Table 2

Comparison of light- and shade-adapted leaves of Müller-Thurgau, Riesling and Dornfelder: Leaf thickness, maximum CO_2 assimilation rate (A_{max}) and stomatal conductance at A_{max} (g at A_{max}), excessive light at 500 µmol m^{-2} s^{-1} , the xanthophyll pool (violaxanthin + antheraxanthin + zeaxanthin), ß-carotene and lutein. (For details: Fig. 2)

	Müller-Thurgau		Riesling		Dornfelder	
Leaf thickness (mm)						
full sun	1.20	(0.15)	1.37	(0.12)	1.51	(0.11)
shade	1.06	(0.18)	1.19	(0.21)	1.31	(0.15)
A_{max} (µmol CO_2 m^{-2} s^{-1})						
full sun	12.70	(0.80)	14.30	(1.50)	16.70	(0.80)
shade	8.80	(1.10)	9.10	(1.20)	11.40	(0.50)
g at A_{max} (mmol H_2O m^{-2} s^{-1})						
full sun	70	(7)	93	(9)	95	(12)
shade	45	(8)	65	(10)	68	(8)
Excessive PAR at 500 µmol m^{-2} s^{-1} (%)						
full sun	31.90	(2.80)	23.10	(2.10)	19.70	(3.20)
shade	67.80	(3.60)	68.30	(4.30)	67.90	(5.00)
Xanthophyll pool (mol mol^{-1})						
full sun	0.55	(0.11)	0.60	(0.18)	0.85	(0.21)
shade	0.19	(0.01)	0.19	(0.02)	0.21	(0.02)
ß-carotene (mol mol^{-1})						
full sun	1.39	(0.29)	1.20	(0.35)	1.44	(0.38)
shade	0.88	(0.21)	0.78	(0.31)	0.82	(0.18)
Lutein (mol mol^{-1})						
full sun	2.40	(0.24)	2.42	(0.10)	2.51	(0.17)
shade	1.71	(0.30)	1.73	(0.13)	1.98	(0.17)

( ) confidence limits at the 5 % level

ties were shown to have relative high rates of ETR (Fig. 2) indicating that they use relatively more energy in photochemical processes. In shade-adapted leaves thermal dissipation capacity reached a plateau already at moderate PAR (Müller-Thurgau and Riesling); only in shade-adapted Dornfelder leaves NPQ continued to increase with PAR up to 2,350 µmol m^{-2} s^{-1} indicating a higher thermal dissipation capacity.

Photoprotective pigments: There is much evidence from literature that NPQ is closely correlated with zeatin (Z), Z being part of the xanthophyll cycle which facilitates dissipation of excess energy via conversion of violaxanthin (V) to antheraxanthin (A) and zeaxanthin (Z) in the thylakoid membrane (Demmig-Adams and Adams 1992, Björkman and Demmig-Adams 1994).

After a high light treatment of light-adapted leaves (3,000 µmol m^{-2} s^{-1} for 5 min to de-epoxidise V to A and Z (Düring 1999)) Z related to the V + A + Z pool was highest in Dornfelder (0.29) followed by Riesling (0.21) and Müller-Thurgau (0.16).

Moreover the V + A + Z pool size itself has been reported to be causally related to the capacity for non-radiative dissipation of energy (Björkman and Demmig-Adams 1994). In light-adapted leaves it was approximately three times

(Müller-Thurgau, Riesling) or 4 times (Dornfelder) higher, than in shade-adapted leaves; comparing light-adapted leaves the pool size of Dornfelder was distinctly higher than that of Riesling or Müller-Thurgau (Tab. 2).

ß-carotene, a component of the pigment-proteine complexes of the thylakoid membranes which has been considered to protect the membrane against over-excitation of the chlorophyll (Siefermann-Harms 1987, Young 1991) and the α-carotene-derived lutein which has recently been shown to be involved in the photoprotective mechanism of PSI and PSII antenna (Pineau et al. 2001), were significantly higher in light-adapted leaves of grape than in shade-adapted leaves; differences between varieties were not significant (Tab. 2).

Excessive light: Another way to study the sensitivity of leaves to high light is to compare the actually observed relative ETR values with responses predicted for a 'model leaf' following the methodical proposals of Schreiber et al. (1994) (for details: Fig. 1). The deviation from the optimal quantum line 'a' can be considered an integrative measure of the light stress produced during the establishment of light response curves.

As expected, the proportion of excessive light was distinctly higher in shade-adapted leaves compared to light-

adapted leaves (Tab. 2). Only light-adapted leaves showed varietal differences, values of excessive light of Müller-Thurgau leaves being significantly higher than those of Riesling and Dornfelder.

It appears that the low rate of excess light in light-adapted Dornfelder leaves is associated with a higher proportion of light energy utilisation in processes of photosynthesis, *e.g.* ETR and CO_2 assimilation, so that less thermal energy needs to be dissipated (Figs 2 and 3, Tab. 2). In addition, the higher xanthophyll pool in light-adapted Dornfelder leaves and its higher capacity to dissipate energy in shade-adapted leaves may have contributed to its higher photoprotection capacity.
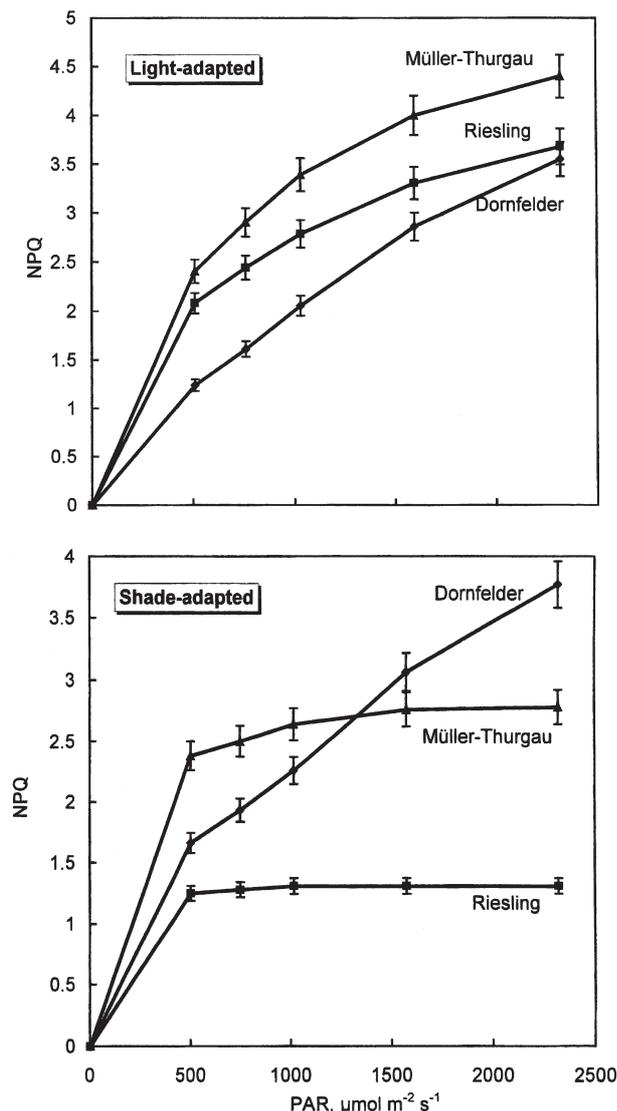

Fig. 3: Non-photochemical quenching (NPQ) of light- and shade-adapted Müller-Thurgau, Riesling and Dornfelder leaves exposed to light intensities increasing from 0 to 2,350 µmol m^{-2} s^{-1}. (For details: Fig. 2)

Conclusion

To the best of our knowledge, for the first time the present results demonstrate that field-grown grapevine varieties differ with regard to their light utilisation and

photoprotection. Further research will have to elucidate the adaptability or tolerance capacity, which is genetically fixed (LICHTENTHALER 1996).

References

ADAMS, W. W.; DEMMIG-ADAMS, B.; LOGAN, B. A.; BARKER, D. H.; OSMOND, C. B.; 1999: Rapid changes in xanthophyll cycle-dependent energy dissipation and photosystem II efficiency in two vines, *Stephania japonica* and *Smilax australis*, growing in the understory of an open *Eucalyptus* forest. Plant Cell Environ. **22**, 125-136.

BILGER, W.; BJÖRKMAN, O.; 1990: Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in *Hedera canariensis*. Photosynt. Res. **25**, 173-185.

BJÖRKMAN, O.; DEMMIG-ADAMS, B.; 1994: Regulation of photosynthetic light energy capture, conversion, and dissipation in leaves of higher plants. In: E. D. SCHULZE, M. M. CALDWELL (Eds.): Ecophysiology of Photosynthesis, 17-47. Springer-Verlag, Berlin.

CAEMMERER, S. VON; FARQUHAR, G. D.; 1981: Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta **153**, 376-387.

DEMMIG-ADAMS, B.; ADAMS, W. W.; 1996: The role of xanthophyll cycle carotenoids in the protection of photosynthesis. Trends Plant Sci. **1**, 21-26.

DÜRING, H.; 1998: Photochemical and non-photochemical responses of glasshouse-grown grape to combined light and water stress. Vitis **37**, 1-4.

- -; 1999: Photoprotection in leaves of grapevines: Responses of the xanthophyll cycle to alterations of light intensity. Vitis **38**, 21-24.

HORTON, P.; RUBAN, A. V.; WALTERS, R. G.; 1996: Regulation of light harvesting in green plants. Ann. Rev. Plant Physiol. Plant Mol. Biol. **47**, 655-684.

LICHTENTHALER, H. K.; 1996: Vegetation stress: An introduction to the stress concept in plants. J. Plant Physiol. **148**, 4-14.

LONG, S. R.; HUMPHRIES, S.; FALKOWSKI, P. G.; 1994: Photoinhibition of photosynthesis in nature. Ann. Rev. Plant Physiol. Plant Mol. Biol. **45**, 633-662.

MAXWELL, K.; JOHNSON, G. N.; 2000: Chlorophyll fluorescence – a practical guide. J. Exp. Bot. **51**, 659-668.

MELIS, A.; 1999: Photosystem-II damage and repair cycle in chloroplasts: What modulates the rate of photodamage *in vivo*? Trends Plant Sci. **4**, 130-135.

NOBEL, P. S.; 1983: Biophysical Plant Physiology and Ecology. W. H. Freeman, San Francisco, USA.

PEARCY, R. W.; 1990: Sunflecks and photosynthesis in plant canopies. Ann. Rev. Plant Physiol. Plant Mol. Biol. **41**, 421-453.

PINEAU, B.; GÉRARD-HIRNE, C.; SELVE C.; 2001: Carotenoid binding to phytosystem I and II of *Chlamydomonas reinhardtii* cells grown under weak light or exposed to intense light. Plant Physiol. Biochem. **39**, 73-85.

SCHREIBER, U.; 1997: Chlorophyllfluoreszenz und photosynthetische Energieumwandlung: Einfache einführende Experimente mit dem TEACHING-PAM Chlorophyll-Fluorometer. H. Walz GmbH (Ed.), Germany.

- -; BILGER, W.; NEUBAUER, C.; 1994: Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of *in vivo* photosynthesis. In: E. D. SCHULZE, M. M. CALDWELL (Eds.): Ecophysiology of Photosynthesis, 49-70. Springer-Verlag, Berlin.

SIEFERMANN-HARMS, D.; 1987: The light harvesting and protective functions of carotenoids in photosynthetic membranes. Physiol. Plant. **69**, 561-568.

Thayer, S. S.; Björkman, O.; 1990: Leaf xanthophyll content and composition in sun and shade determined by HPLC. Photosynt. Res. **23**, 331-343.

Trebst, A.; 1994: Dynamics in photosystem. II. Structure and function. In: E. D. Schulze, M. M. Caldwell (Eds.): Ecophysiology of Photosynthesis, 3-16. Springer-Verlag, Berlin.

Vogelmann, T. C.; 1993: Plant tissue optics. Ann. Rev. Plant Physiol. Plant Mol. Biol. **44**, 231-251.

Young, A. J.; 1991: The photoprotective role of carotenoids in higher plants. Physiol. Plant. **83**, 702-708.