Survey of Thermal Energy Dissipation and Pigment Composition in Sun and Shade Leaves

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A survey was conducted of the magnitude of energy dissipation in photosystem II (expressed as nonphotochemical quenching of chlorophyll fluorescence, NPQ) as well as leaf carotenoid composition of a wide range of different plant species growing in deep shade and/or full sun. Consistently higher levels of the reversible component of NPQ as well as higher degrees of rapidly attainable de-epoxidation of the xanthophyll cycle (VAZ) pool were observed in sun leaves compared to deep shade leaves. It is concluded that these altered features of the xanthophyll cycle allowed sun leaves to dissipate excess energy more effectively over the short term. In addition to the rapid increase in reversible NPQ, shade leaves exhibited a slow further, and sustained, increase in NPQ. In contrast to these deep shade leaves experimentally exposed to high PFDs, understory leaves experiencing highly variable PFD in their natural environment appeared to be able to dissipate excess excitation energy adequately via xanthophyll cycle-dependent thermal dissipation. Furthermore, very consistent trends across plant species were observed for changes in carotenoid composition (pools of carotenes, VAZ, and other xanthophylls) in response to light environment, as long as it is assumed that in some species β-carotene can be replaced by α-carotene and in a few plant species lutein can be replaced by lactucaxanthin. Sun leaves consistently exhibited much greater levels of VAZ than shade leaves but very similar levels of lutein and neoxanthin (all on a Chl basis) as well as an only slightly higher ratio of total carotenes to the pool of all xanthophylls.

Key words: Carotenoids — Chlorophyll fluorescence — Photosystem II — Sun and shade leaves — Xanthophyll cycle.

Protection of photosynthesis from light stress in the natural environment is provided via the thermal dissipation of excess energy in photosystem II (Demmig-Adams and Adams 1996c), a process requiring the presence of the xanthophyll cycle components zeaxanthin and antheraxanthin (Z+A) as well as a low intrathylakoid pH (for recent reviews see Demmig-Adams and Adams 1996a, Demmig-Adams et al. 1996, Horton et al. 1996, Eskling et al. 1997, Gilmore 1997). A greater pool size of the xanthophyll cycle (VAZ) in sun versus shade leaves has been reported consistently for a number of plant species (Thayer and Björkman 1990, Osmond et al. 1993, Demmig-Adams and Adams 1992, 1994, Brugnoli et al. 1994, Demmig-Adams et al. 1995, Adams and Demmig-Adams 1996, Logan et al. 1996). Less, as well as less consistent, information is available concerning the ability of sun and shade leaves to perform energy dissipation in PSII. While some studies showed correlations between the level of energy dissipation and aspects of VAZ pool size and/or conversion state (Osmond et al. 1993, Demmig-Adams and Adams 1994, Brugnoli et al. 1994, Demmig-Adams et al. 1995, Adams and Demmig-Adams 1996), others reported examples where no such correlation was found (Johnson et al. 1993). In addition, it has recently been suggested that the level of energy dissipation can be independent of the PSII antenna size (Gilmore et al. 1996, Gilmore 1997) and therefore possibly also independent of the ratio of Z (or Z+A) to Chl.

The present study reports a survey of the magnitude of thermal energy dissipation in PSII (as NPQ) as well as the carotenoid composition of leaves of a wide range of different plant species growing in deep shade and/or full sunlight. The results of the present survey show a correlation between the level of thermal dissipation in PSII antennae and the conversion state of the xanthophyll cycle across species, provided that only the reversible component of NPQ was considered. It was also clear that shade leaves exhibited a considerable, albeit slow, further and sustained increase in NPQ beyond the rapid increase in reversible NPQ. In addition, a consistent trend across species was observed for changes in the various components of the carotenoid fraction in response to light environment. Also examined was the in situ response of shade leaves that experienced bright sunflecks in their natural environment as is very typical for leaves growing in the understory of a forest.

Material and Methods

Plant species and growth environments—All plants were growing outside in the ground on the campus of the University of

Colorado in Boulder during the summer of 1993 and sampled between 14 and 24 June. Leaves were chosen that were exposed to full sunlight (1,500–2,000 μmol photons m^{-2} s^{-1}) at midday. Species growing in full sun included *Aquilegia coerulea* James [Colorado Columbine], *Brassica* sp. [Mustard], *Carduus* sp. [Thistle], *Fraxinus* sp. [Ash; seedling stage], *Iris* sp. [Iris], *Lactuca serriola* L. [Prickly Lettuce], *Malva neglecta* Wallr. [Mallow], *Medicago sativa* L. (Alfalfa), *Plantago major* L. [Common Plantain], *Rosa* sp. [Rose], *Syringia vulgaris* L. [Lilac], *Sonchus oleraceus* L. [Annual Sow-thistle], *Taraxacum officinale* G.H. Weber [Common Dandelion], *Tulipa* sp. (Tulip), *Urtica* sp. [Nettle], and *Viburnum lantana* L. [Wayfaring Tree]. Species growing in deep shade included *Euonymus kiautschovicus* Loesener, *Parthenocissus quinquefolia* (L.) Planch. [Virginia Creeper], *Viola* sp. [Violet], and *Hedera helix* L. [English Ivy]. Deeply shaded leaves received no direct sunflecks due to self-shading and/or shading by buildings or tree trunks. Species growing in both full sun and deep shade included *Mahonia repens* (Lindley) Don [Creeping Holly] and *Vinca minor* L. [Periwinkle]. Plant species growing in the understory and monitored in situ during a sunfleck included *Hedera helix*, *Mahonia repens*, *Parthenocissus quinquefolia*, *Viola* sp., and *Vinca minor*.

Pigment analyses—Carotenoids were analysed by HPLC (see Adams and Demmig-Adams 1992) using the column and gradient system described by Gilmore and Yamamoto (1991). Triplicate samples were usually taken from each leaf. Error bars in the figures show the standard error for n=18 (species in the sun) or n=7 (species in deep shade). The presence of lactucaxanthin (dihydroxy-ε,ε-carotene) in the genera *Euonymus* and *Lactuca* was established previously (Phillip and Young 1995, Demmig-Adams and Adams 1996b). While whole leaf discs were analyzed for pigment content, fluorescence was obtained from the upper leaf surface only. This nevertheless appears to allow an appropriate comparison of pigment and fluorescence characteristics, presumably due to the fact that most of the leaf's chloroplasts are present in the upper, palisade portion of the leaf.

Chlorophyll fluorescence—Measurements of Chl fluorescence in situ during sunflecks in the field were performed with a portable PAM-2000 fluorometer (Walz, Effeltrich, Germany) as described previously (Demmig-Adams and Adams 1996c) and during experimental exposure of excised leaves or leaf discs to high PFDs in an oxygen electrode chamber (see Demmig-Adams et al. 1990). All fluorescence measurements in the chamber were preceded by a 10-min incubation of leaves at 25°C under far-red light in the absence of actinic light after which time the control values of the initial and maximal yields of fluorescence, F_o and F_m, were determined (see van Kooten and Snel 1990 for fluorescence nomenclature). This was followed by illumination with 1,800–2,050 μmol photons m^{-2} s^{-1} of white light for 10–15 min unless otherwise indicated. After this time, the actinic light was again replaced with far-red illumination to determine the minimal level of F_o'. During the entire time while leaves were kept in the electrode, a slow stream of humidified air was passed over them.

Results

Kinetics of changes in the level of energy dissipation (NPQ) in sun and shade leaves—Fig. 1 shows a time course of changes in the level of energy dissipation in PSII (NPQ) upon an experimental exposure of sun and deep shade leaves of *Vinca minor* to a high PFD. The sun leaf exhibited a greater level of NPQ that was reached rapidly

over the first 10–15 min of exposure than the shade leaf. The portion of NPQ attained over 15 min of high light that was reversible within an additional 16 min in darkness was 3.82 in the sun leaf versus only 1.90 in the shade leaf. In addition, the pool size of the xanthophyll cycle on a Chl basis as well as the degree of conversion to Z and A at the end of the 15 min-exposure to high light were greater in the sun leaf (100 mmol VAZ per mol Chl; (A+Z)/(V+A+Z)= 0.81) compared to the shade leaf (28 mmol VAZ per mol Chl; (A+Z)/(V+A+Z)=0.44; see Table 1).

In addition, Fig. 1 illustrates that over longer periods
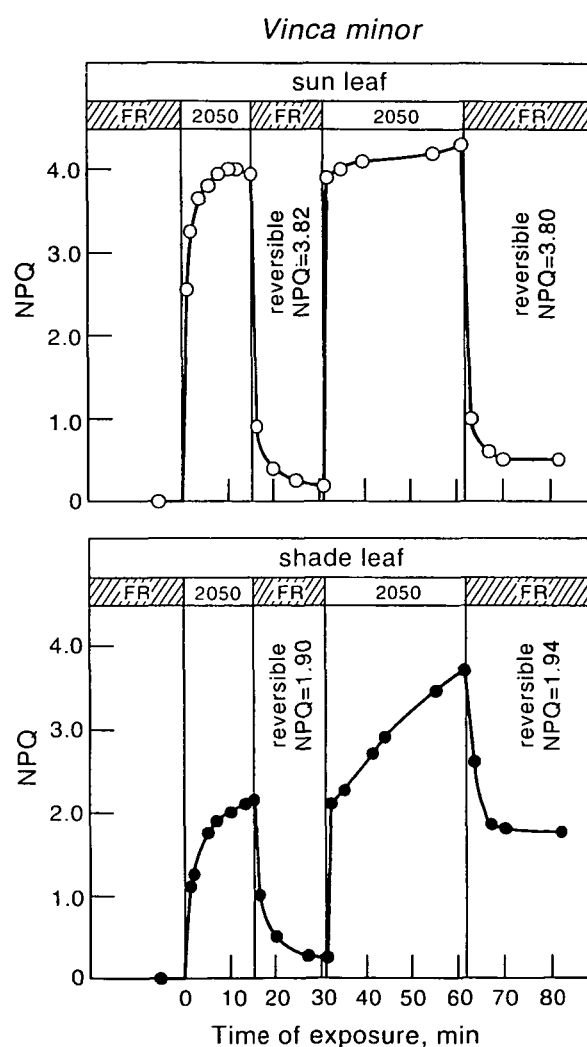
Vinca minor

Fig. 1 Time course of changes in the level of thermal energy dissipation in PSII (NPQ) upon exposure to a high PFD of 2,050 μmol photons m^{-2} s^{-1} for leaves from populations of *Vinca minor* growing in full sun or deep shade, respectively. The reversible component of NPQ was obtained by subtracting the sustained component of NPQ ($=F_m/F_m rec-1$ where $F_m rec$ is the partially recovered F_m after 15–20 min of recovery under far-red light) from total NPQ during high light ($=F_m/F_m'-1$).
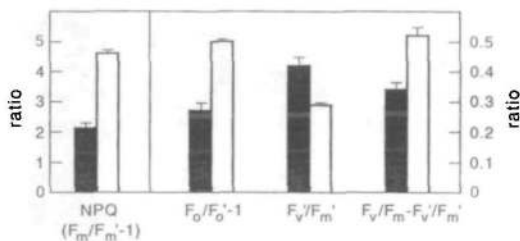
Table 1 Chl fluorescence characteristics after 10–15 min at PFDs of 1,800–2,050 μmol photons m^{-2} s^{-1} in leaves of sun and/or shade populations of different plant species

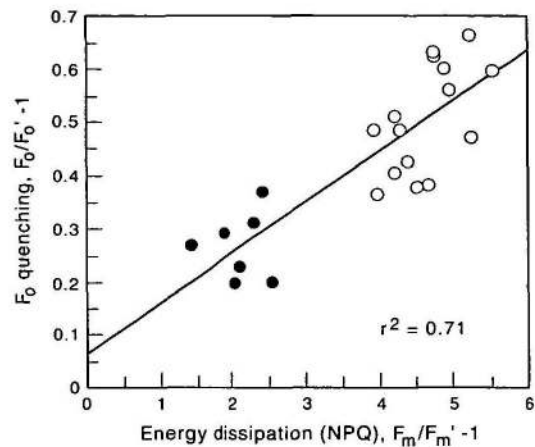|	NPQ of F_m'	NPQ of F_o'	F_v'/F_m'	F_v/F_m $-F_v/F_m'$	Chl (μmol m^{-2})	Chl a/b	VAZ	βC	aC	L	La	N	Z	Z+A
							(mmol (mol Chl)$^{-1}$)						(per VAZ pool)	
Full sun														
Aquilegia	5.0				264	3.31	118	134	0	182	—	44	0.67	0.80
					±29	±0.35	±7	±12	±0	±14		±7	±0.02	±0.03
Brassica	4.3	0.48	0.29	0.52	363	3.31	72	118	0	180	—	42	0.67	0.78
					±19	±0.09	±6	±1	±0	±9		±2	0.01	±0.01
Carduus	4.5	0.38	0.30	0.53	359	3.46	86	114	0	142	—	33	0.68	0.79
Lactuca	4.2	0.40	0.30	0.51	288	3.17	117	126	0	118	54	44	0.72	0.79
					±13	±0.29	±7	±19	±0	±7		±4	±0.02	±0.01
Fraxinus	4.7	0.63	0.30	0.51	297	3.38	126	121	4	163	—	43	0.65	0.83
Iris	3.9	0.48	0.33	0.47	461	3.07	87	117	0	176	—	44	0.68	0.77
					±66	±0.07	±10	±10	±0	±10		±3	±0.02	±0.01
Mahonia	5.2	0.47	0.26	0.57	428	3.47	90	128	1	150	—	47	0.52	0.66
					±61	±0.31	±9	±8	±1	±10		±10	±0.05	±0.05
Malva	4.0	0.36	0.36	0.47	442	3.69	138	145	0	163	—	43	0.72	0.82
					±45	±0.52	±6	±12	±0	±14		±5	±0.05	±0.03
Medicago	4.2	0.51	0.30	0.50	400	3.21	85	116	0	166	—	38	0.62	0.78
Plantago	5.2	0.66	0.29	0.50	319	3.51	87	130	0	172	—	38	0.71	0.82
Rosa	4.4				297	3.63	103	133	1	153	—	43	0.69	0.80
					±23	±0.05	±9	±7	±1	±14		±2	±0.01	±0.01
Sonchus	4.4	0.43	0.30	0.51	334	3.45	95	118	0	108	77	38	0.70	0.79
Syringia	4.9	0.56	0.25	0.56	251	3.94	152	133	8	191	—	38	0.64	0.81
Taraxacum	4.9	0.60	0.30	0.51	275	3.24	95	122	3	171	—	40	0.71	0.82
					±25	±0.24	±1	±4	±3	±8		±3	±0.01	±0.01
Tulipa	4.3				596	2.91	84	124	0	158	—	42	0.52	0.66
					±41	±0.22	±5	±7	±0	±12		±3	±0.02	±0.02
Urtica	4.7	0.38	0.29	0.54	337	3.42	110	139	0	158	—	40	0.70	0.84
Viburnum	5.5	0.60	0.22	0.59	508	3.54	120	106	6	165	—	38	0.58	0.74
Vinca	4.7	0.63	0.32	0.49	375	3.46	100	108	0	165	—	38	0.66	0.81
Deep shade														
Euonymus	1.4	0.27	0.54	0.22	495	2.39	39	68	24	137	55	49	0.44	0.51
					±49	±0.07	±1	±4	±3	±2	±4	±2	±0.01	±0.01
Euonymus	1.9	0.29	0.44	0.31	460	2.70	37	58	39	79	78	36	0.33	0.42
					±38	±0.05	±2	±2	±6	±4	±6	±3	±0.01	±0.02
Hedera	2.3	0.31	0.35	0.39	747	2.36	37	69	20	165	—	43	0.35	0.42
					±11	±0.05	±1	±3	±0	±1		±1	±0.04	±0.03
Mahonia	2.1	0.20	0.41	0.36	462	2.29	36	72	18	199	—	50	0.46	0.54
					±45	±0.10	±1	±6	±1	±5		±6	±0.03	±0.01
Parthenocissus	2.4	0.37	0.40	0.36	398	2.33	48	41	37	155	—	42	0.24	0.33
					±39	±0.06	±2	±6	±1	±6		±1	±0.01	±0.01
Viola	2.6	0.20	0.38	0.39	339	2.28	49	94	0	177	—	56	0.53	0.63
					±28	±0.02	±1	±3	±0	±2		±4	±0.00	±0.01
Vinca	2.1	0.23	0.46	0.33	521	2.58	28	87	0	161	—	38	0.34	0.44
					±23	±0.02	±1	±4	±0	±6		±1	±0.04	±0.04

Carotenoid and Chl contents and composition are also shown. Chl fluorescence characteristics include the levels of thermal energy dissipation in PSII antennae (NPQ of $F_m'=F_m/F_m'-1$), levels of F_o' quenching (NPQ of $F_o'=F_o/F_o'-1$), the efficiency of open PSII units (F_v'/F_m'), and the change in this latter efficiency that was induced by the high PFD compared to the dark state ($F_v/F_m-F_v'/F_m'$). All data are means of two or three (±SD) leaves per plant species. aC = a-carotene, βC = β-carotene, Chl = sum of Chl $a+b$, La = lactucaxanthin, L = lutein, N = neoxanthin, VAZ = sum of violaxanthin (V) + antheraxanthin (A) + zeaxanthin (Z). Most plant species did not contain lactucaxanthin (indicated as — in column for La).

of time, further slow increases in NPQ were observed in the shade leaf, in which the quickly attainable level of NPQ had been relatively low. In contrast, the sun leaf did not exhibit any marked further increases in NPQ beyond the rapidly attained, high initial levels of NPQ. In addition, the high level of NPQ of about 4 in the sun leaf continued to be largely rapidly reversible even after longer periods of exposure. In contrast, in the shade leaf only the initial, relatively small increase in NPQ was rapidly reversible while it appeared that the secondary slow increases in NPQ were not (Fig. 1).

Survey of relationship between rapidly reversible NPQ (as well as other PSII characteristics) and the xanthophyll cycle—Sun and deep shade leaves of a range of different plant species were subjected to exposures similar to the ones shown in Fig. 1 to determine the degree of rapidly reversible changes in NPQ and other PSII characteristics after short exposures of 10 to 15 min. Table 1 shows a complete list of various PSII fluorescence characteristics as well as pigment levels and composition for all leaves. For greater ease of comparison, Fig. 2 shows pooled data for all shade leaves versus all sun leaves, indicating consistently greater levels of energy dissipation in PSII in sun versus shade leaves. The Stern-Vollmer quenching of F_m' and F_o' was roughly proportional, as would be expected (after Kitajima and Butler 1975) for energy dissipation in the antennae (Fig. 2, 3; see also Gilmore and Yamamoto 1993, Demmig-Adams and Adams 1996c). Furthermore, the efficiency of open PSII units during illumination (given by the ratio of variable to maximum fluorescence, F_v'/F_m') decreased more in sun versus shade leaves (Fig. 2). A decrease in this PSII efficiency—that has also been referred to as the 'excitation capture efficiency of PSII' (Schreiber et al. 1994)—is typically associated with increases in the level of energy
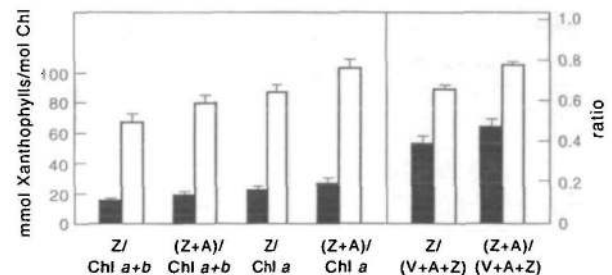

Fig. 3 Relationship between the level of thermal energy dissipation in PSII (i.e. NPQ of $F_m = F_m/F_m' - 1$) and the level of quenching of initial fluorescence ($F_o/F_o' - 1$) as a result of exposures to a high PFD for sun and deep shade leaves. Data points represent means (n = 2 or 3) from seven plant species growing in deep shade (closed circles) and 18 plant species growing in full sunlight (open circles). Leaves were exposed to PFDs of 1,800–2,050 μmol photons $m^{-2} s^{-1}$ for 10–15 min.

dissipation in PSII antennae (Demmig-Adams et al. 1995, Gilmore and Björkman 1995, Demmig-Adams and Adams 1996c). Thus the observed greater decreases in PSII efficiency are consistent with the greater increases in reversible NPQ in sun leaves compared to shade leaves. Fig. 2 furthermore shows the change in PSII efficiency, expressed as difference between the efficiency of open PSII units in darkness (F_v/F_m) and during illumination (F_v'/F_m'), that was also greater in sun versus shade leaves. All these data indicate significantly greater levels of rapidly attainable ther-

Fig. 2 Level of thermal energy dissipation in PSII (i.e. NPQ of $F_m = F_m/F_m' - 1$), level of quenching of initial fluorescence ($F_o/F_o' - 1$), efficiency of open PSII units during illumination (F_v'/F_m'), and change in this efficiency ($F_v/F_m - F_v'/F_m'$) as a result of exposures to a high PFD for sun and deep shade leaves. Data represent means ($\pm$SE) of means (n = 2 or 3) from seven plant species growing in deep shade (black bars) versus 18 plant species growing in full sunlight (white bars). Leaves were exposed to PFDs of 1,800–2,050 μmol photons $m^{-2} s^{-1}$ for 10–15 min. All sun leaves showed dark-adapted, pre-exposure levels of F_v/F_m of 0.8 or above and shade leaves showed levels of 0.77–0.79.

Fig. 4 Degree of de-epoxidation of the xanthophyll cycle as a result of exposures to a high PFD for sun and deep shade leaves. The degree of de-epoxidation was expressed either as the level of zeaxanthin (Z) or zeaxanthin + antheraxanthin (Z + A) relative to either Chl $a + b$, Chl a, or the total pool size of the xanthophyll cycle (V + A + Z). Data represent means ($\pm$SE) of means (n = 2 or 3) from seven plant species growing in deep shade (black bars) versus 18 plant species growing in full sunlight (white bars). Leaves were exposed to PFDs of 1,800–2,050 μmol photons $m^{-2} s^{-1}$ for 10–15 min.
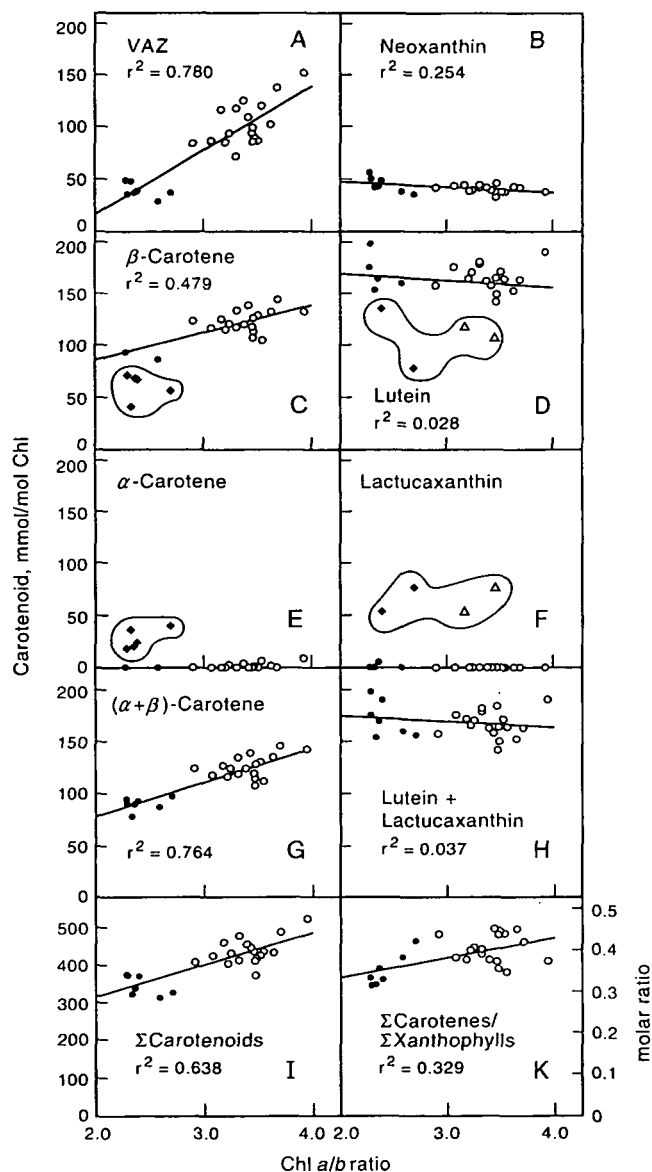
mal energy dissipation in PSII antennae in sun versus shade leaves during these 10- to 15-min exposures to high PFDs.

In addition, analyses of the xanthophyll cycle conver-

Fig. 5 Relationship between the Chl a/b ratio and levels of (A) the xanthophyll cycle (VAZ) pool, (B) neoxanthin, (C) β-carotene, (D) lutein, (E) α-carotene, (F) lactucaxanthin, (G) the sum of α-carotene and β-carotene, (H) the sum of lutein and lactucaxanthin, (I) the sum of all carotenoids, and (K) the molar ratio of all carotenes to all xanthophylls for sun and deep shade leaves. All carotenoid pools are expressed on a Chl basis. Data points represent means (n=2 or 3) from seven plant species growing in deep shade (filled symbols) and 18 plant species growing in full sunlight (open symbols). In C and E the filled diamonds represent plant species that possessed α-carotene when growing in the shade, and in D and F the filled diamonds and open triangles represent species possessing lactucaxanthin.
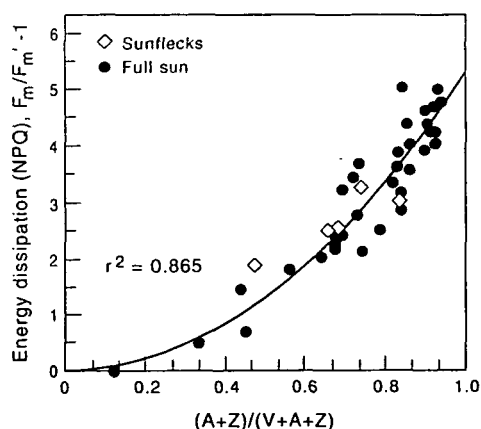
sion state (Fig. 4; see Table 1 for data from each species) indicate that sun leaves also underwent a greater degree of conversion of the xanthophyll cycle to its de-epoxidized forms Z and A than shade leaves during these treatments. This conclusion remains the same independent of whether Z or Z+A were considered and whether they were expressed on a Chl $a+b$, Chl a, or VAZ pool basis (Fig. 4).

Survey of changes in carotenoid composition in response to natural light environment—Table 2 shows pooled data for all sun leaves versus all deep shade leaves for the Chl a/b ratio, Chl and VAZ pools on a leaf area basis as well as ratios of carotenoids to Chl and relative to one another (see Table 1 for individual data). Sun leaves possessed higher Chl a/b ratios and levels of VAZ per area than shade leaves, and had similar or perhaps slightly lower Chl contents per area. In addition, on a Chl basis sun leaves exhibited considerably greater pools of VAZ and β-carotene, similar pools of lutein and neoxanthin and lower levels of α-carotene (see Table 1 for individual data) than shade leaves.

Fig. 5 shows the individual components of the carotenoid fraction on a Chl basis, i.e. VAZ (Fig. 5A), neoxanthin (Fig. 5B), β-carotene (Fig. 5C), lutein (Fig. 5D), α-carotene (Fig. 5E), and lactucaxanthin (Fig. 5F) in leaves with different Chl a/b ratios. This approach allows a direct comparison of carotenoid levels and composition of widely diverse plant species but is not intended to imply a functional relationship between Chl a/b ratio and carotenoid composition. Consistent trends among all plant species considered were observed for changes in carotenoid composition if it was assumed that β-carotene can be replaced by α-carotene in some plant species (diamonds in Fig. 5C,

Fig. 6 Relationship between the de-epoxidation state of the xanthophyll cycle, (A+Z)/(V+A+Z), and the level of thermal energy dissipation in PSII (NPQ) for leaves of five plant species growing in the understory and monitored during sunflecks (open diamonds) as compared to leaves of a large variety of plant species growing and monitored in full sun (filled circles). Data for the full sun-exposed leaves are from Demmig-Adams and Adams (1996c).

Table 2 Differences in chlorophyll and carotenoid composition from pooled data for all sun leaves versus all shade leaves ($\pm$SD)

	Shade leaves	Sun leaves	Sun, % of shade
Chl a/b ratio	2.42 ± 0.16	3.40 ± 0.24	$+41\%$
μmol Chl m^{-2}	489 ± 129	366 ± 92	-25%
μmol VAZ m^{-2}	19 ± 4	37 ± 10	$+95\%$
mmol ΣCar (mol Chl)$^{-1}$	346 ± 27	437 ± 35	$+26\%$
mmol VAZ (mol Chl)$^{-1}$	39 ± 7	104 ± 21	$+167\%$
mmol βC (mol Chl)$^{-1}$	70 ± 18	124 ± 11	$+77\%$
mmol $a+\beta$C (mol Chl)$^{-1}$	90 ± 6	125 ± 11	$+39\%$
mmol L (mol Chl)$^{-1}$	153 ± 38	160 ± 21	$+5\%$
mmol (L+La) (mol Chl)$^{-1}$	173 ± 17	167 ± 13	-4%
mmol N (mol Chl)$^{-1}$	45 ± 7	41 ± 3	-9%
carotenes xanthophylls^{-1}	0.35 ± 0.04	0.40 ± 0.03	$+14\%$

n$=7$ for number of species found in the shade and n$=18$ for species found in the sun. Each species is represented by the mean of either two or three samples from shade and/or sun leaves. $a+\beta$C$=$sum of a- and β-carotene, βC$=\beta$-carotene, Chl$=$sum of Chl $a+b$, ΣCar$=$sum of all carotenoids, La$=$lactucaxanthin, L$=$lutein, N$=$neoxanthin, VAZ$=$sum of violaxanthin (V)$+$antheraxanthin (A)$+$zeaxanthin (Z).

E) and lutein replaced by lactucaxanthin in others (diamonds and triangles in Fig. 5D, F). These species-independent trends consisted of strong increases in the VAZ pool (Fig. 5A), increases in the sum of $(a+\beta)$-carotene (Fig. 5G), but virtually no change in lutein ($+$lactucaxanthin; Fig. 5H) or neoxanthin (Fig. 5B) per Chl with increasing Chl a/b ratios (see also Table 2). The most notable difference between sun and shade leaves was thus the greater level of VAZ in sun leaves. The sum of all carotenoids showed an increase (Fig. 5I) that could be accounted

for mainly by the increases in VAZ (Fig. 5A) and $(a+\beta)$-carotene (Fig. 5G). The sum of all carotenoids increased by $+91$ mmol (mol Chl)$^{-1}$ (for the means of sun versus shade leaves; Table 2) from the increases by $+65$ mmol (mol Chl)$^{-1}$ in VAZ pool and by $+35$ mmol (mol Chl)$^{-1}$ in $(a+\beta)$-carotene pool, with negligible decreases in the means for sun vs. shade for the pools of lutein$+$lactucaxanthin (-6 mmol (mol Chl)$^{-1}$) and neoxanthin (-4 mmol (mol Chl)$^{-1}$) (Table 2). It is clear that the ratio of carotenes (a- and β-carotene) to xanthophylls (VAZ$+$L$+$La$+$N)
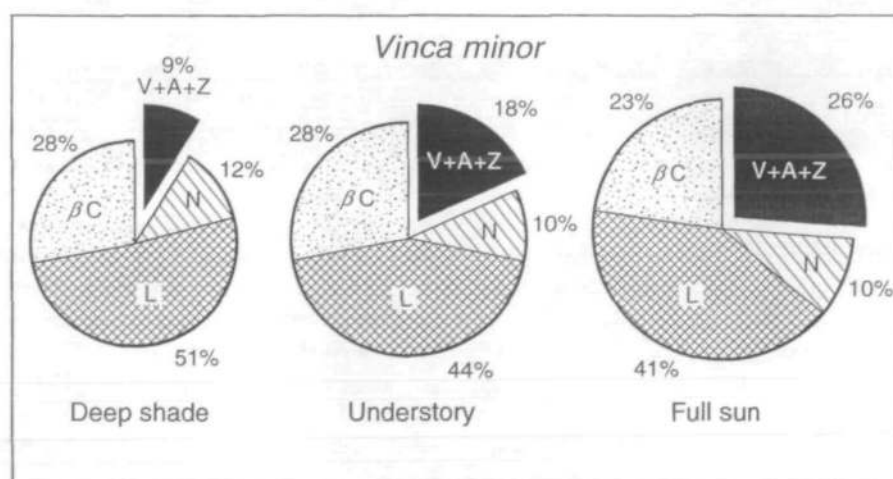
Fig. 7 Carotenoid composition of leaves from deep shade, understory (open shade with sunflecks), and sun populations of *Vinca minor*. The total size of the pies represents the relative size of the total carotenoid pool per Chl. Further indicated are the fractions of the total carotenoid pool that were constituted by the xanthophyll cycle pool (V$+$A$+$Z), neoxanthin (N), lutein (L), and β-carotene (βC). Data are means of three leaves from each growth environment.

was only slightly higher in sun leaves compared to shade leaves (Fig. 5K; see also Table 2).

Xanthophyll cycle-dependent energy dissipation in leaves acclimated to understory environments with highly variable PFD—Since deep shade leaves were found to have a lesser propensity for rapid increases in thermal energy dissipation in PSII, it was investigated whether shade leaves growing in the understory and receiving intermittent exposure to high PFD showed evidence of insufficient energy dissipation in PSII in the field as well. Fig. 6 shows the relationship between thermal energy dissipation in PSII (NPQ) and the conversion state of the xanthophyll cycle for a range of sun and understory leaves in situ in their natural environment at peak exposure to sunlight. Peak exposure occurred for the sun leaves considered at around midday and during brief periods of several min duration each for the shade leaves in the understory. It is apparent that the relationship between energy dissipation and xanthophyll cycle conversion state was similar for sun leaves and understory leaves during natural sun exposure (Fig. 6). This would suggest that understory leaves use the same mechanism of energy dissipation during rapid changes in PFD, i.e. zeaxanthin-dependent thermal dissipation, as sun leaves during continuous sun exposure.

From these results, it was expected that shade leaves from understory environments with highly variable PFDs might possess larger xanthophyll cycle pools than leaves that had developed in deep shade. Fig. 7 shows that this was indeed the case, i.e. there was a gradual increase in VAZ pool from *Vinca minor* leaves growing in deep shade to those receiving brief exposures to high PFDs every day and to those growing in full sun exposure.

Discussion

Differences between populations of plants found naturally at the extremes of the irradiance spectrum (sun and deep shade) with respect to the carotenoid composition of their leaves and its functional significance—What is the significance of differences in the VAZ pool size?—When sun and deep shade populations of a large number of plant species were compared with respect to their carotenoid composition, several clear and species-independent trends emerged. Sun leaves growing with ample supply of sunlight showed higher Chl *a/b* ratios than shade leaves. This is commonly interpreted as an indication of a higher ratio of core to light-harvesting (CP and LHC) complexes in sun leaves, for the purpose of shifting emphasis away from light-harvesting and towards higher rates of photochemistry (see discussion in Anderson and Osmond 1987). Another way of assessing the ratio of core complexes to CPs and LHCs is from the ratio of carotenes to xanthophylls since carotenes are thought to be bound to core complexes whereas xanthophylls are predominantly bound to CPs and

LHCs (Yamamoto and Bassi 1996). Surprisingly, the large data base of deep shade versus full sun-acclimated leaves analysed here shows only slightly higher ratios of carotenes to xanthophylls for sun versus shade leaves with different Chl *a/b* ratios. In addition, while the VAZ pool on a Chl basis was much greater in sun versus shade leaves, the levels of lutein (+lactucaxanthin) and neoxanthin (also per Chl) were very similar.

Within PSII antennae, VAZ is thought to be enriched in the minor CPs (CP24, CP26, CP29) but occurs also in LHCII, lutein is bound to CPs and LHCs, and neoxanthin predominantly to LHCII (Yamamoto and Bassi 1996). Lactucaxanthin is thought to replace lutein in its binding sites predominantly in LHCII (Phillip and Young 1995). The acclimation pattern described above suggests that it may not be a change in the overall ratio of core complexes to light-harvesting complexes that is causing the much greater ratio of VAZ to Chl in sun leaves. Instead it is possible that the stoichiometry of Chl *b* (see also Tanaka and Melis 1997) and/or VAZ bound to a given protein complex may vary. In sun leaves more VAZ might be bound to one or more of the light-harvesting proteins than in shade leaves.

Very consistent trends among plant species emerge for the shade-sun acclimation of carotene and xanthophyll levels (see Fig. 5), provided that it is assumed that in some plant species *a*-carotene can replace *β*-carotene (consistent with the results of Benson and Cobb 1981, Siefermann-Harms 1984, Young and Britton 1989, Thayer and Björkman 1990, Demmig-Adams et al. 1995, Königer et al. 1995, Demmig-Adams and Adams 1996b) and in a few others lactucaxanthin can replace lutein (consistent with Phillip and Young 1995, Demmig-Adams and Adams 1996b). Lactucaxanthin (dihydroxy-*ε,ε*-carotene) is an isomer of lutein (dihydroxy-*β,ε*-carotene) and zeaxanthin (dihydroxy-*β,β*-carotene). Lactucaxanthin is restricted to a few taxa and occurs, in addition to *Euonymus kiautschovicus*, in several genera in the tribe Cichoriae of the Asteraceae where it has been characterized particularly in *Lactuca sativa* (Siefermann-Harms et al. 1981, Phillip and Young 1995).

What is the significance of the greater Z+A levels that can be attained rapidly in sun leaves?—Does the enrichment of the xanthophyll cycle components relative to Chl and the other carotenoids have functional significance for the ability to dissipate excitation energy as well? This has been somewhat unclear since some studies found correlations between the levels of Z+A on either a Chl basis or per VAZ pool and the levels of NPQ (Demmig-Adams and Adams 1992, 1994, Osmond et al. 1993, Brugnoli et al. 1994, Demmig-Adams et al. 1995, Adams and Demmig-Adams 1996) while others reported no such correlation for some plant species (Johnson et al. 1993), and most recently the functional relevance of the ratio of Z/Chl has been questioned (Gilmore et al. 1996, Gilmore 1997). Gilmore et al. (1996) reported that Chl-deficient mutants preferentially

accumulating the PSII core and inner antennae but not the major, peripheral LHCII, and consequently exhibiting very high ratios of xanthophyll cycle components to Chl, nevertheless showed similar levels of energy dissipation as the wildtype. This is consistent with our own observation that young, bright yellow leaves of Chl-deficient mutants (Golden privet) had 10-fold higher levels of Z or Z+A on a Chl basis (Demmig-Adams et al. 1997) but the same level of NPQ compared with older, greener leaves (Adams, W.W. III and Demmig-Adams, B., unpublished data). The conversion state of the xanthophyll cycle $(A+Z)/(V+A+Z)$ was, however, similarly high in the yellow versus green leaves.

In the present study we observed a consistent and species-independent association between the VAZ pool as well as its conversion state and the reversible portion of NPQ that can be induced rapidly upon exposure to high irradiance and reverses equally rapidly upon return to low light (see Fig. 2, 4). This strong association among VAZ pool size, rapidly attainable Z+A levels and rapidly reversible NPQ in sun versus shade leaves can be interpreted in a number of ways; it may be that NPQ is indeed dependent on Z+A level over a wide range of Z+A concentrations. Alternatively, if—as Gilmore (1997) suggests—a few strategically placed Z+A per PSII are sufficient for maximal energy dissipation, the total pool of VAZ or Z+A may influence parameters such as e.g. the occupation of the quenching site.

It is important to note that, while deep shade leaves were unable to show large increases in rapidly induced and reversible NPQ, they did show pronounced secondary and slowly reversible increases in NPQ (Fig. 1). While it is often assumed that slowly reversible NPQ may be related to photoinhibitory damage, there are numerous reports of slowly reversible NPQ that is associated with retention of Z+A (see Demmig-Adams et al. 1998 and references therein). It is thus possible that the difference between deep shade and sun leaves with respect to energy dissipation in PSII concerns mainly kinetic properties and reversibility, with only sun leaves being capable of both rapid and strong increases and decreases in energy dissipation level.

Ability of understory leaves to cope with excess excitation in their natural environment—Understory leaves that regularly experienced brief exposures to high PFDs in their natural environment underwent a partial sun-acclimation with respect to the pool size of the xanthophyll cycle that was intermediate between that of leaves growing in deep shade versus full sun (Fig. 7; see also Demmig-Adams et al. 1995, Königer et al. 1995, Logan et al. 1996, 1997). The similarity of the relationship between xanthophyll cycle conversion state and either the level of energy dissipation (Fig. 6) or PSII efficiency F_v'/F_m' (Demmig-Adams et al. 1995) in these understory leaves suggests that energy dissipation in shade leaves is xanthophyll cycle-dependent as it is

in sun leaves. In nature the xanthophyll cycle and the ability for energy dissipation thus appear to adjust continuously to increases in light stress encountered (see also Demmig-Adams et al. 1995). Therefore, the lesser ability of leaves grown in deep shade without sunflecks to respond to experimental exposures with rapid increases in reversible NPQ (Fig. 1, 2) does not appear to reflect a shortfall under natural conditions.

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