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Photosynthesis, xanthophylls, and D1 phosphorylation under winter stress

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Introduction

Plants that maintain green tissues during the winter all employ the xanthophyll cycle in photoprotection, albeit to very different degrees. Woody evergreen species in sun-exposed sites maintain their photosynthetic apparatus in a highly dissipative and photoprotected state throughout the day and night, with a large fraction of the xanthophyll cycle retained as zeaxanthin and antheraxanthin (Z+A) and sustained low PSII efficiency F_v/F_m (Adams *et al*. 2001). This occurs initially in response to low temperatures, but a certain proportion of this sustained (Z+A)-dependent energy dissipation becomes "locked in" as the winter progresses, remains engaged during intermittent periods of warmer weather, and takes days to reverse upon transfer to darkness or low light at room temperature. In contrast, herbaceous mesophytes such as spinach and the weed *Malva neglecta* exhibited only relatively minor nocturnally-sustained Z+A retention and lowered PSII efficiency exclusively during periods with subfreezing temperature, and this was rapidly reversible within minutes upon warming. In the following, further distinguishing features between these two groups of species are identified.

Materials and methods

Light- and CO_2-saturated rates of oxygen evolution (gross photosynthetic capacity) at 25°C were ascertained according to Björkman & Demmig 1987. Leaf discs for analysis of pigments (Adams & Demmig-Adams 1992), carbohydrates (Schulze *et al.* 1991; Hendrix 1993), and proteins (Ebbert *et al.* 2001) were collected and immediately frozen in liquid nitrogen. Chlorophyll fluorescence was determined as described in Demmig-Adams *et al.* (1996).

Results and Discussion

Contrasting Acclimatory Responses.

Between August and January, photosynthetic capacity doubled in *Malva*, with no significant differences in foliar chlorophylls and carotenoid levels (not shown), the xanthophyll cycle pool, nor in the diurnal conversion state of the xanthophyll cycle and the level of energy dissipation (both integrated from predawn to dusk) between the two days (Fig. 1; the integrated diurnal incident PFD in January was 56% of that in August). In contrast, sun leaves of *Vinca minor* exhibited a several-fold decrease in photosynthetic capacity and in

chlorophyll and carotenoid content per area (not shown), but an increase in the xanthophyll cycle carotenoids relative to chlorophyll, and an integrated diurnal xanthophyll cycle conversion and dissipation of absorbed excitation of almost 100% in the winter versus only 60% and less than 50%, respectively, in the summer (Fig. 1). On the other hand, *Vinca* growing in the shade exhibited acclimatory characteristics more similar to *Malva* than *Vinca* growing in the sun (Fig. 1), with a photosynthetic capacity in winter more than twice that observed in the summer, and xanthophyll cycle characteristics and integrated dissipation similar in the winter compared to the summer.

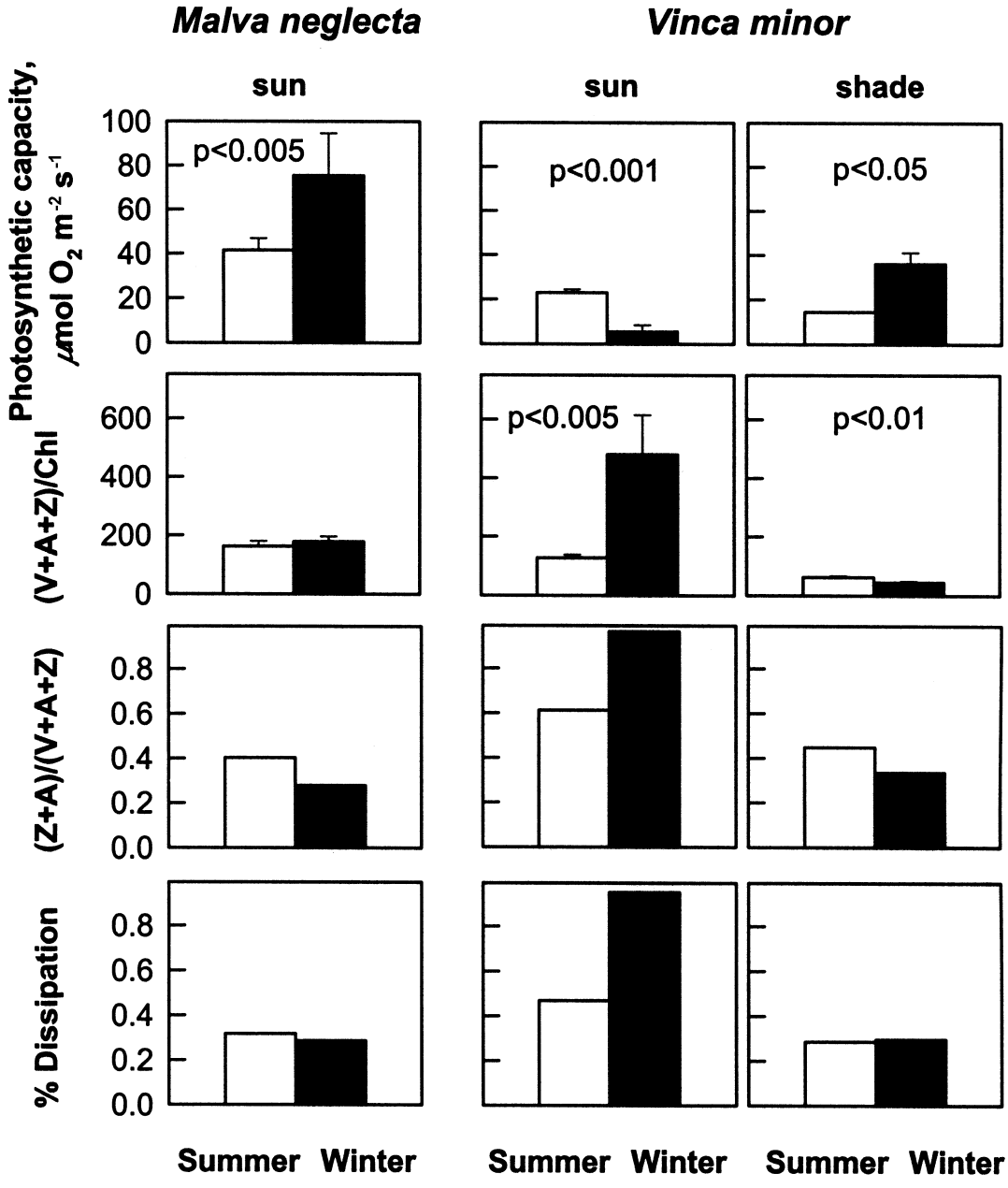

Fig. 1. Photosynthetic capacity, V+A+Z per Chl *a*+*b*, integrated diurnal xanthophyll cycle conversion state, and integrated diurnal 1-F_v/F_m (for the percentage of absorbed excitation dissipated thermally) in sun *Malva* as well as sun and shade *Vinca*. *Malva* was characterized on 15 August 1997 and 20 February 1996 (for photosynthetic capacity) and 22-23 August 1997 and 29-30 January 1998. *Vinca* was characterized on 25 June 1997 and 10 February 1998. Values are mean±SD, n=3, and degree of significance was determined by Student's *t*-test. Data from Verhoeven *et al.* (1999), Adams *et al.* (2001), and unpublished data.

For both species, foliar soluble sugar content was higher in the winter than in the summer (Fig. 2), especially in *Vinca* growing in the sun (and even more so in the shade, unpublished data). Pronounced accumulation of foliar starch between predawn and dusk (and hence nocturnal removal) was observed in both species during the summer (Fig. 2). However, during the winter, diurnal accumulation of starch and nocturnal removal was only observed in *Malva* (Figs. 2 and 3). All of these data suggest that *Malva*, a biennial herbaceous species that exhibits growth of new leaves during warm periods in the winter, maintains or upregulates photosynthetic capacity and continues to synthesize and utilize carbohydrates on a daily basis when temperatures permit enzymatic functioning on milder winter days. In contrast, sun-exposed populations of the sclerophyte *Vinca* (but not shade populations) appear to enter a state of minimal photosynthetic activity throughout the winter.

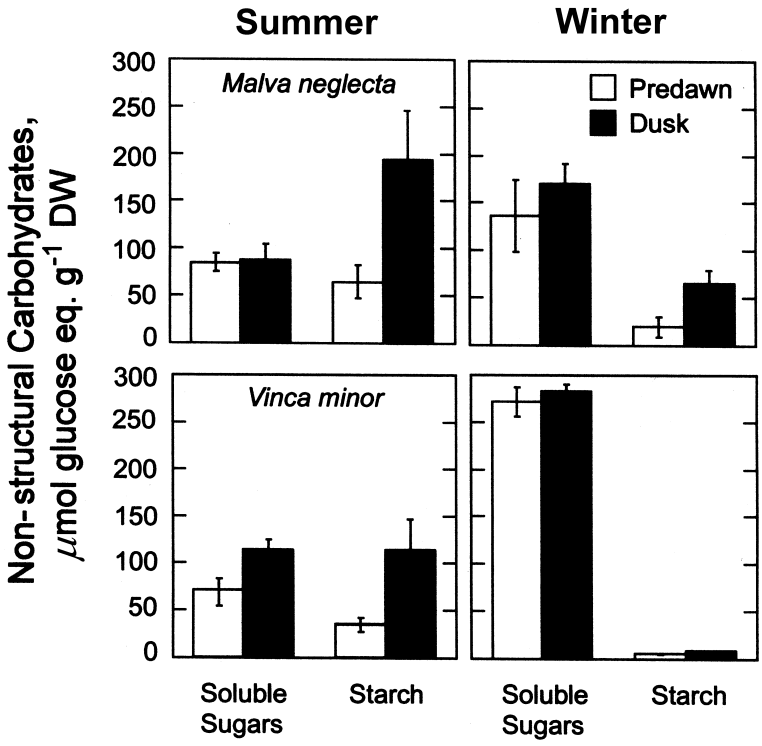

Fig. 2. Predawn and end of day pool sizes of foliar soluble sugars and starch in sun-exposed populations of *Malva* and *Vinca* in summer versus winter. *Malva* was characterized on 22-23 August 1997 and 29-30 January 1998, and *Vinca* on 28 August 1997 and 10 February 1998. Values are mean±SD, n=3

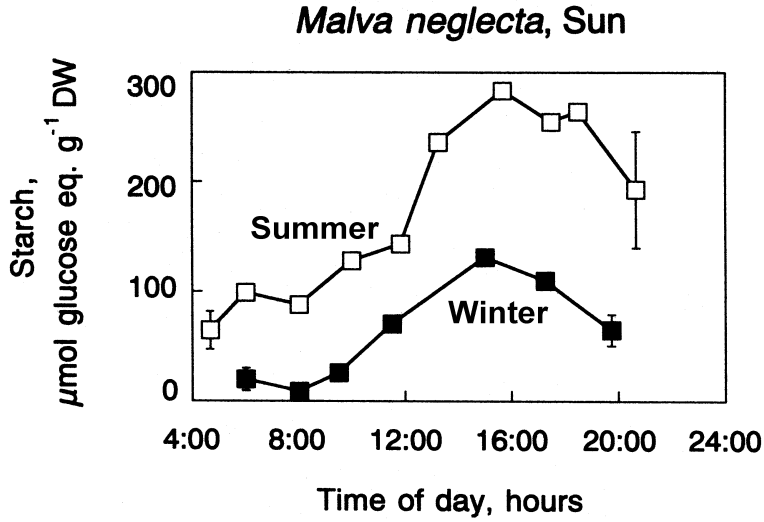

Fig. 3. Diurnal changes in total foliar starch content in *Malva* on 22-23 August 1997 and 29-30 January 1998.

The generality of these responses was explored through additional characterization of seasonal differences in photosynthetic capacity in *Malva* and three other species (Table 1). In both sun-exposed mesophytes, photosynthesis was lowest in late summer and elevated again in winter. In the sun-exposed conifers, the response was similar to that observed in *Vinca*, with the lowest rates of photosynthesis observed in winter. Shade Ponderosa pine exhibited elevated photosynthesis in winter, similar to shade *Vinca*, whereas shade Douglas fir exhibited low photosynthesis rates in winter.

Table 1. Photosynthetic capacity (μmol O_2 m^{-2} s^{-1}; mean±SD, n=6 or greater) in leaves or needles from plants growing in either full sunlight or in deep shade. Early Summer = 24 May to 16 June 2000, Late Summer = 7-15 August 2000, and Winter = 10-22 January 2001. Most plants growing in Gregory Canyon, west of Boulder, CO.

	Early Summer	Late Summer	Winter
SUN-EXPOSED MESOPHYTES			
Malva neglecta	76±11	31±3	66±13
Verbascum thapsus (Mullein)	33±6	21±9	37±18
SUN-EXPOSED CONIFERS			
Pinus ponderosa (Ponderosa pine)	36±3	29±5	21±4
Pseudotsuga menziesii (Douglas fir)	26±5	17±4	3±3
SHADED CONIFERS			
Pinus ponderosa (Ponderosa pine)	24±4	11±5	26±6
Pseudotsuga menziesii (Douglas fir)	13±2	8±3	2±1

Association Between Sustained D1 Protein Phosphorylation and Sustained (Z+A)-Dependent Eenergy Dissipation.

We have recently described an additional feature in overwintering leaves concerning the phosphorylation pattern of the D1 protein of the PSII reaction center (Adams *et al.* 2001). In overwintering leaves a portion of the D1 protein pool remains phosphorylated nocturnally (as indicated by anti-phosphothreonine antibody) until predawn on cold winter nights (Fig. 4). The degree of D1 protein phosphorylation

varied greatly between *Malva* and the conifer Douglas fir. In *Malva*, the total level of D1 relative to Chl remained constant between winter and summer and the level of non-phosphorylated D1 did not decrease appreciably in the winter. This suggests that no more than a low percentage of the total D1 pool remained phosphorylated predawn in *M. neglecta* that also showed only a small degree of Z+A retention and a small sustained F_v/F_m depression. In contrast, the total D1 levels per Chl declined somewhat in the winter in Douglas fir and the levels of the non-phosphorylated form of D1 decreased dramatically, suggesting that a major fraction of the D1 pool remains phosphorylated on cold winter nights in overwintering Douglas fir needles in which the xanthophyll cycle pool remained highly converted to Z+A until predawn and predawn F_v/F_m remained at very low levels. Separation of the non-phosphorylated and phosphorylated forms of D1 and use of an additional D1 antibody that immunoreacts with both forms confirmed that a large fraction of the D1 pool remained phosphorylated on this cold night in Douglas fir (data not shown).

What is the Switch that Triggers Engagement of Xanthophyll-dependent Energy Dissipation?

Energy dissipation requires not only xanthophylls but also the PsbS protein (Li *et al.* 2000). PsbS-deficient mutants do not show a major structural rearrangement of the thylakoid membrane normally observed in high light. The level of PsbS per total Chl remained constant in *Malva* between summer and winter, but was higher in the winter compared to the summer in Douglas fir (data not shown). Yet another protein, PsbZ, has been implicated in energy dissipation since PsbZ-deficient mutants (grown at lower temperature), while forming large amounts of Z+A, showed diminished levels of energy dissipation and of PSII protein (particularly D1) phosphorylation (Swiatek *et al.* 2001). The engagement of xanthophyll-dependent energy dissipation may thus also involve a structural change of the PSII core, possibly related to D1 phosphorylation, and consistent with correlations between sustained D1 phosphorylation and (Z+A)-dependent energy dissipation (see also Ebbert *et al.* 2001). A comprehensive model for the switch that engages xanthophyll-dependent energy dissipation would therefore feature a major structural change in the thylakoid membrane involving PsbS and PsbZ, and protonation (Gilmore 1997) and phosphorylation events.

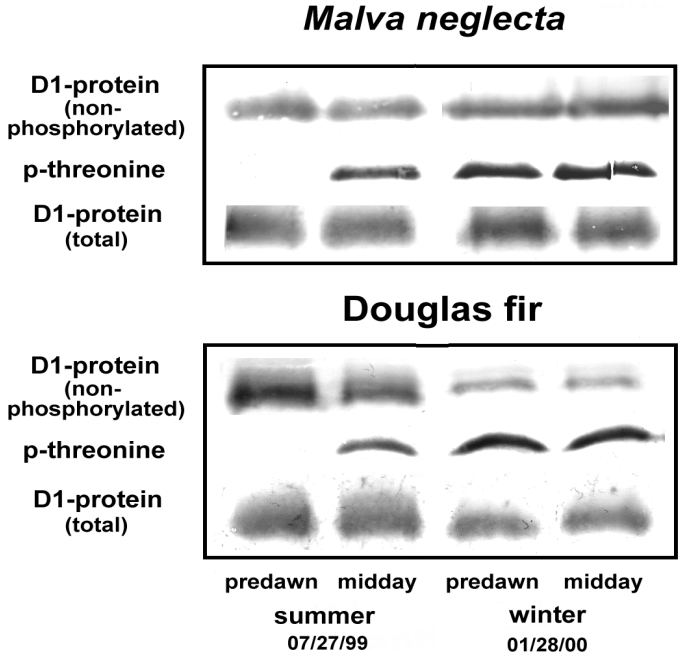

Fig. 4. Western blots (equal chlorophyll basis) showing the predawn and midday level of non-phos-phorylated D1, phosphorylated D1 using an anti-phosphothreonine anti-body, and the total D1 protein pool in Malva leaves and Douglas fir needles on 27 July 1999 (predawn and midday air temperatures of 20°C and 34°C, respectively) and 28 January 2000 (predawn and midday air temperatures of –7°C and 0°C, respectively).

In *Yucca* plants in the hot and dry Mojave desert summer, nocturnal Z+A retention was accompanied by only small decreases in F_v/F_m, whereas in the colder winter season there was a more pronounced effect on F_v/F_m (Fig. 5). This was interpreted as a lesser degree of nocturnal engagement of the retained Z+A in energy dissipation at higher temperatures versus lower temperatures (Barker *et al.* 2002). It is likely that in (Z+A)-retaining *Yucca* leaves exhibiting the more pronounced decreases in PSII efficiency (F_v/F_m) at lower temperatures, PSII (D1) was more highly phosphorylated, since the activity of PSII protein phosphatase is inhibited when its regulator protein becomes attached to the thylakoid membrane at lower temperatures (Rokka *et al.* 2000). Further investigations of a possible role of D1 phosphorylation in the engagement of energy dissipation at low temperatures are thus warranted.

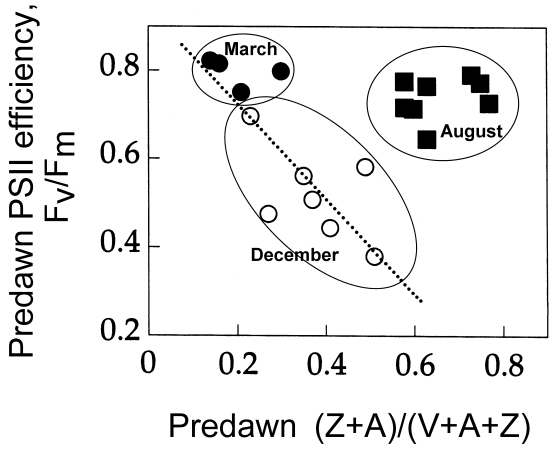

Fig. 5. Relationship between the xanthophyll cycle conversion state and the light capture efficiency of open PSII units ascertained predawn from leaves of *Yucca brevifolia* and *Y. schidigera* on a hot day (7 August 1993), on a cold day (16 December 1993), and a moderate day (22 March 1994), both growing in the Mojave desert. From Barker *et al*. (2002).

Acknowledgments

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References

Adams WW III, Demmig-Adams B (1992) *Planta* **186**, 390-398.
Adams WW III, Demmig-Adams B, Rosenstiel TN, Ebbert V (2001) *Photosynthesis Research* **67**, 51-62.
Barker DH, Adams WW III, Demmig-Adams B, Logan BA, Verhoeven AS, Smith SD (2002) *Plant, Cell and Environment* **25**, in press
Björkman O, Demmig B (1987) *Planta* **170**, 489-504.
Demmig-Adams B, Adams WW III, Barker DH, Logan BA, Verhoeven AS, Bowling DR (1996) *Physiologia Plantarum* **98**, 253-264.
Ebbert V, Demmig-Adams B, Adams WW III, Mueh KE, Staehelin LA (2001) *Photosynthesis Research* **67**, 63-78.
Gilmore AM (1997) *Physiologia Plantarum* **99**, 197-209.
Hendrix DL (1993) *Crop Science* **33**, 1306-1311.
Li X-P, Björkman O, Shih C, Grossman AR, Rosenquist M, Jansson S, Niyogi KK (2000) *Nature* **403**, 391-395.
Rokka A, Aro E-M, Herrmann RG, Andersson B, Vener AV (2000) *Plant Physiology* **123**, 1525-1535.
Schultze W, Stitt M, Schultze E-D, Neuhaus HE, Fichter K (1991) *Plant Physiology* **95**, 890-895.
Swiatek M, Kuras R, Sokolenko A, Higgs D, Olive J, Cinque G, Müller B, Eichacker LA, Stern DB, Bassi R, Herrmann RG, Wollman F-A (2001) *The Plant Cell* **13**, 1347-1367.
Verhoeven AS, Adams WW III, Demmig-Adams B (1999) *Oecologia* **118**, 277-287.