New Phytologist

Review

Research review

Sustained energy dissipation in winter evergreens

Author for correspondence:
Amy Verhoeven
Tel: +1 651 962 5278
Email: asverhoeven@stthomas.edu

Received: *11 June 2013*
Accepted: *27 July 2013*

Amy Verhoeven

Biology Department, University of St Thomas, Saint Paul, MN 55015, USA

New Phytologist (2014) **201:** 57–65
doi: 10.1111/nph.12466

Key words: conifers, energy dissipation, low-temperature stress, nonphotochemical quenching of chlorophyll fluorescence (NPQ), zeaxanthin.

Summary

Evergreens are faced with the challenge, during winter, of low temperatures in combination with light exposure, resulting in an imbalance between light absorption and its utilization via photosynthetic carbon reduction. To cope with excess light, evergreens increase their use of thermal energy dissipation, which occurs in a sustained form during winter. There are two forms of sustained thermal dissipation that occur in winter-stressed evergreens, characterized by their rate of reversal upon warming. A rapid form reverses within minutes to hours upon warming, while a slower form reverses over the course of days. The light environment and the severity of winter conditions both play a role in determining the relative amount of each type of sustained energy dissipation throughout the winter. It is suggested that the two forms of sustained dissipation observed in winter-stressed evergreens correspond to sustained forms of the two mechanisms of thermal energy dissipation proposed by Holzwarth and colleagues, with the rapidly reversible component corresponding to a sustained form of the energy-dependent form of thermal energy dissipation (qE) and the slowly reversible component corresponding to a sustained form of the zeaxanthin-dependent mechanism (qZ). Additional outstanding questions and future directions are discussed.

Introduction

Evergreens growing in seasonally cold environments maintain their leaves during harsh winter climates. This strategy allows for some flexibility, relative to deciduous species, in the timing of winter-induced down-regulation of photosynthesis and the reversal of these processes in the spring. However, it results in the exposure of leaves or needles to extremely low temperatures in combination with light, both of which fluctuate on a daily and seasonal scale. Evergreen trees cease growth and become dormant during winter, undergoing a process of acclimation to cold temperatures, triggered by both shortening photoperiod and decreasing temperatures (Levitt, 1980; Bigras *et al.*, 2001). Additionally, temperature effects on the enzymatic reactions of photosynthetic carbon reduction result in little to no carbon fixation occurring during winter months. Low temperatures do not preclude light absorption, and thus evergreens are faced with a severe imbalance between light absorption and its utilization via carbon reduction and other metabolic pathways (Öquist & Huner, 2003). As a result, evergreens must up-regulate photoprotective processes that either dissipate excess absorbed excitation energy or provide protection from damaging reactions that occur during these conditions.

In general, plants utilize a variety of strategies to protect the photosynthetic apparatus in conditions when light absorption exceeds the capacity for utilization of the products of the light reactions. These include mechanisms to decrease light absorption (e.g. leaf or chloroplast movements), thermal dissipation of excess absorbed light, and enzymatic pathways of reactive oxygen scavenging (the water–water cycle) and the photorespiratory pathway (for a recent review, see Takahashi & Badger, 2011). In winter-stressed evergreens, where low temperatures inhibit enzymatic reactions, the primary photoprotective mechanism appears to be increases in thermal dissipation of excess absorbed light.

Thermal dissipation in nonwinter conditions

In unstressed plants, thermal energy dissipation is a dynamic process that becomes engaged in conditions of excess light to safely dissipate excess absorbed excitation energy and protect the photosynthetic apparatus from damage. This process reverses

quickly when light becomes limiting, allowing maximal photochemical efficiency. The engagement of thermal energy dissipation results in a decrease in Chl fluorescence yield, which can be measured as nonphotochemical quenching of Chl fluorescence (NPQ; Bilger & Björkman, 1990).

The engagement of thermal dissipation is regulated by three components: the buildup of a proton gradient across the thylakoid membrane (ΔpH; see Table 1 for glossary of terms used within this review), the conversion of the xanthophyll pigment violaxanthin (V) via antheraxanthin (A) into zeaxanthin (Z), and the protonation of the photosystem II (PSII) subunit S (PsbS; Müller *et al.*, 2001; Murchie & Niyogi, 2011). The process is regulated by the pH of the thylakoid lumen such that in conditions of excess light, which result in a high ΔpH across the thylakoid membrane, the enzyme violaxanthin de-epoxidase is activated and catalyzes the conversion of V to Z. Additionally, the protein PsbS functions as a sensor of lumen pH that is necessary for the induction of energy dissipation (Li *et al.*, 2004). The precise molecular mechanisms giving rise to thermal energy dissipation are under some debate (for reviews, see Horton, 2012; Jahns & Holzwarth, 2012; Ruban *et al.*, 2012). The disengagement of thermal energy dissipation occurs in limiting light conditions when the ΔpH is reduced, Z is converted to V, and PsbS is no longer protonated. The flexible nature of the engagement and disengagement of thermal dissipation is critical in allowing plants to cope with changes in light in combination with other environmental conditions, and allow for a balance between optimization of photosynthesis and protection of the photosynthetic apparatus (Demmig-Adams & Adams, 2006; Demmig-Adams *et al.*, 2012).

The Chl fluorescence parameter NPQ, used to monitor thermal energy dissipation, has been subdivided into components, defined by the relaxation kinetics upon darkening leaves that were previously exposed to excessive light (Müller *et al.*, 2001; Nilkens *et al.*, 2010). These components have recently been redefined, based on a study examining a variety of NPQ mutants in *Arabidopsis*, to include the rapidly reversible (qE), the slowly reversible (qZ) and the very slowly reversible (qI) components of energy dissipation (Nilkens *et al.*, 2010). The qE component, which is the major component in most conditions, responds quickly to changes in light intensity (engages and disengages within seconds to minutes). This component has been shown to be dependent upon the ΔpH and PsbS, and also requires synthesis of Z (Dall'Osto *et al.*, 2005; Nilkens *et al.*, 2010). The rapid relaxation of qE upon darkening is dependent upon the dissipation of the ΔpH. The qZ component of NPQ is slower in its engagement (10–30 min) and disengagement (10–60 min). This component is dependent upon accumulation of Z, but its maintenance is independent of both ΔpH and PsbS (Dall'Osto *et al.*, 2005; Nilkens *et al.*, 2010). The slower relaxation of qZ upon darkening is dependent upon the enzymatic conversion of Z to A and V (Nilkens *et al.*, 2010). The qI component of NPQ has been characterized as being very slowly reversible (> 120 min), is present in relatively stressful environmental conditions, and occurs more frequently in plants adapted to such conditions (Müller *et al.*, 2001; Nilkens *et al.*, 2010).

A study using ultrafast fluorescence on intact leaves identified two quenching sites for NPQ probably involving two mechanisms of thermal energy dissipation and corresponding to the qE and qZ components of NPQ (Holzwarth *et al.*, 2009). The quenching site associated with qE, termed Q1, is associated with the major light-harvesting trimers (LHCII) that become detached from the PSII reaction center supercomplex (Johnson *et al.*, 2011). The study suggests that Q1 quenching is modulated

Table 1 Glossary of terms used within this review

CP24	Minor light-harvesting antennae protein, also called Lhcb6
CP26	Minor light-harvesting antennae protein, also called Lhcb5
CP29	Minor light-harvesting antennae protein, also called Lhcb4
ELIP	Early light-induced protein, family of proteins related to light-harvesting proteins that are involved in stress responses
Energy dissipation	A process whereby excitation energy absorbed by Chl is dissipated thermally. Commonly measured using the Chl fluorescence parameter NPQ
F_v/F_m	Photochemical efficiency of photosystem II. This is a Chl fluorescence parameter that measures the maximal efficiency of photosynthesis in dark-acclimated plants. Typical values for unstressed plants are in the range 0.8–0.85
LHC	Light-harvesting complex. This is a general abbreviation referring to light harvesting-proteins associated with photosystem II (PSII) (Lhcb1–6) or those associated with PSI (Lhca1–4). Note that the PSII light-harvesting complexes include the major LHCII trimers, which are a combination of Lhcb1–3, and the minor LHC proteins, which exist as monomers (Lhcb4–6)
NPQ	Nonphotochemical quenching of Chl fluorescence. This is a Chl fluorescence parameter that measures the decrease in yield of Chl fluorescence as a result of thermal energy dissipation
qE	The component of NPQ that reverses rapidly (seconds to minutes) upon darkening a leaf that has been exposed to high light
qI	The component of NPQ that reverses very slowly (hours) upon darkening a leaf that has been exposed to high light. This component is usually only present under extreme stressors
qZ	The component of NPQ that reverses between 10 and 30 min upon darkening a leaf that has been exposed to high light
Q1 quenching	A type of fluorescence quenching proposed by Holzwarth and colleagues that occurs within LHCII trimers that have disassociated from the PSII core and that requires PsbS, a ΔpH and Z (zeaxanthin)
Q2 quenching	A type of fluorescence quenching proposed by Holzwarth and colleagues that occurs within the minor LHC antennae associated with PSII and that requires Z but can occur independently of ΔpH and PsbS
ΔpH	A gradient in pH across the thylakoid membrane that occurs when plants are undergoing photosynthesis
PsbS	A protein in the light-harvesting family of proteins that has been shown to be essential in the process of thermal energy dissipation

by the ΔpH, and requires PsbS, but does not strictly require Z accumulation; however, Z plays a role in modulation of this type of quenching (Holzwarth *et al.*, 2009). The quenching site associated with qZ, termed Q2, was shown to be dependent upon Z, but independent of PsbS and the ΔpH (provided Z is already present, as its formation requires the ΔpH; Dall'Osto *et al.*, 2005). This type of quenching is thought to occur within the minor antennae that remain attached to PSII, which includes three LHC proteins designated CP24, CP26 and CP29 (also denoted Lhcb6, Lhcb5 and Lhcb4, respectively; Holzwarth *et al.*, 2009; Jahns & Holzwarth, 2012).

Sustained thermal dissipation in overwintering evergreens

In overwintering evergreens, the process of thermal energy dissipation changes from the dynamic form that occurs in summer to a sustained engagement of energy dissipation that does not respond to fluctuating light (Öquist & Huner, 2003; Adams *et al.*, 2004). The winter-induced sustained energy dissipation seems to be critical for maintaining the balance between light absorption and its reduced utilization as a result of low-temperature effects on photosynthetic carbon reduction. The transformation of energy dissipation characteristics that occur upon transition from summer to winter involves changes in xanthophyll cycle dynamics as well as changes in the composition and characteristics of the photosynthetic apparatus. This results in a functional change from 'light-harvesting centers', which maximize efficiency of light harvesting and energy transfer during the summer months, to 'dissipating centers' that maximize safe thermal dissipation of absorbed light during the winter months (Öquist & Huner, 2003).

Early studies examining Chl fluorescence of overwintering evergreens found reductions, during winter, in the maximal photochemical efficiency (measured predawn as the Chl fluorescence parameter F_v/F_m; e.g. Öquist & Ögren, 1985). These low F_v/F_m values were shown to correlate with dark-retention of the xanthophyll pigments A and Z (e.g. Adams & Demmig-Adams, 1994). Experiments examining recovery of F_v/F_m upon transferring winter-stressed evergreens into room temperature and low light found that F_v/F_m recovers up to unstressed values over the course of several days (e.g. Ottander & Öquist, 1991), and that recovery correlates closely with the conversion of Z to V (Verhoeven *et al.*, 1996, 1998). Such studies led to the hypothesis that winter reductions in photochemical efficiency are caused by sustained engagement of thermal energy dissipation (e.g. Verhoeven *et al.*, 1996, 1998). A study examining Chl fluorescence spectra at 77 K and lifetime distributions of Chl fluorescence in leaves of the evergreen snow gum, during winter compared with summer, demonstrated changes in both parameters that were consistent with dark-sustained energy dissipation in winter-stressed leaves (Gilmore & Ball, 2000).

Photoprotective pigments in winter-stressed evergreens

Comparisons of leaf pigment concentrations in summer and winter have been conducted in multiple evergreen species in both sun and shade environments, and data relating to photoprotective pigments from five published studies are summarized in Table 2 (Adams & Demmig-Adams, 1994; Ottander *et al.*, 1995; Verhoeven *et al.*, 1998, 2005, 2009). A general response to subzero conditions during winter is that xanthophyll cycle dynamics are arrested, such that light-dependent interconversions of the pigments do not occur.

Table 2 Compilation of pigment data relating to xanthophyll cycle composition ((A + Z)/(V + A + Z)), total amount of xanthophyll pigments (V + A + Z), and lutein content in summer (S) compared with winter (W)

	AZ/(VAZ)		V + A + Z			Lutein		
Species	S	W	S	W	%	S	W	%
Pinus sylvestris[1]	*c.* 0.2	*c.* 0.9	0.025	0.057	+100%	0.055	0.10	+82%
Pinus ponderosa[2]	0.43	0.85	223	274	+23%	384	532	+81%
Pseudotsuga menziesii[2]	0.68	0.90	218	387	+78%	460	555	+20%
Picea pungens[2]			128	272	+112%	251	376	+50%
Euonymus kiautschovicus[3] (sun)	0.85	0.85	47	88	+87%			
E. kiautschovicus[3] (shade)	0	0.62	22	44	+100%			
Taxus x media[4] (sun)	0.85	0.85	114	188	+65%	174	280	+61%
Taxus x media[4] (shade)	0	0.75	49	73	+49%	124	187	+51%
Abies balsamea[5] (sun)	0.60	0.91	68	152	+123%	144	314	+118%
A. balsamea[5] (shade)	0.09	0.76	55	70	+30%	135	213	+58%
Pinus strobus[5]	0.39	0.88	82	133	+63%	148	280	+90%

A, antheraxanthin; V, violaxanthin; Z, zeaxanthin.
For some species data from both sun and shade are reported. The percentage change in winter relative to summer is shown in the column marked %. The units for which total xanthophyll pigments and lutein are reported vary and are indicated below.
[1]Ottander *et al.* (1995), Scots pine (*P. sylvestris*), pigment units in g m^{-2}, 'winter' values are from March.
[2]Adams & Demmig-Adams (1994), Ponderosa pine (*P. ponderosa*), Douglas fir (*Pseudotsuga menziesii*) and Blue spruce (*Picea pungens*), pigment units in nmol (g DW)$^{-1}$.
[3]Verhoeven *et al.* (1998), *E. kiautschovicus*, pigment units in μmol m^{-2}.
[4]Verhoeven *et al.* (2005), *T. x media*, pigment units in μmol (mol Chl)$^{-1}$.
[5]Verhoeven *et al.* (2009), Balsam fir (*A. balsamea*), Eastern white pine, (*P. strobus*), pigment units in μmol (mol Chl)$^{-1}$.

The xanthophyll cycle pigments are maintained in a highly de-epoxidized form (often measured as (A + Z)/(V + A + Z)), with winter values reported in the studies quoted in the range of 0.8–0.9 for leaves collected in sunny conditions and 0.6–0.8 for leaves collected in shaded conditions (Table 2). Additionally, the pool size of the xanthophyll cycle (V + A + Z) increases in winter conditions in most species observed, with winter increases reported ranging from 20 to > 100% above summer values. The same studies have shown that concentrations of lutein increase consistently in winter-stressed leaves (20 to > 100% higher in winter, Table 2). Lutein also has a photoprotective function in plants, with a particular role in quenching the triplet state of Chl (recently reviewed in Jahns & Holzwarth, 2012). The increased concentrations of the VAZ pool and lutein suggest that a component of acclimation to winter stress is increasing the relative content of photoprotective pigments within the photosynthetic apparatus.

Components of sustained thermal energy dissipation

In winter-stressed evergreens, it is not possible to determine an accurate value of the fluorescence parameter NPQ ($F_m/F_m' - 1$), because this value relies on a maximal fluorescence reading (F_m) that is in a relaxed (unquenched) state, usually measured predawn. In low-temperature conditions, the F_m value remains quenched after a full night of darkness. Therefore NPQ is a not a useful measure of energy dissipation in winter-stressed evergreens. To compensate for this problem, some studies have used F_m values measured in summer to calculate winter NPQ (Porcar-Castell, 2011), or estimated winter F_m values by using a value for F_o multiplied by a factor of 5 (Ensminger *et al.*, 2004). Another strategy, which avoids estimating NPQ during winter, is to monitor predawn F_v/F_m values of evergreens, and values below those of unstressed plants (e.g. < 0.80) are indicative of sustained energy dissipation.

One of the standard methods of examining NPQ in unstressed plants is to monitor the kinetics of the reversal of NPQ upon darkening leaves, leading to the identification of the three NPQs described previously. This type of measurement is not possible in winter-stressed evergreens; however, it is possible to examine the reversal of sustained energy dissipation by monitoring increases in F_v/F_m upon removing winter-stressed leaves to room temperature and low light. Such studies have demonstrated that the kinetics of this recovery consists of two phases that differ in relaxation rate, with a rapid phase occurring within minutes to hours of warming, and a slower phase that occurs over several days (Fig. 1a, Ottander & Öquist, 1991; Ottander *et al.*, 1995; Verhoeven *et al.*, 1996, 1998, 2009; Verhoeven, 2013). Both phases correlate with the reconversion of V to Z, suggesting that two mechanisms may be involved in maintaining winter-sustained energy dissipation (Verhoeven *et al.*, 1996, 1998). Thus winter-induced sustained energy dissipation appears to involve at least two components that can be distinguished by their relaxation rate upon warming. These two components will be referred to as the 'rapidly reversible' and the 'slowly reversible' components of sustained energy dissipation.

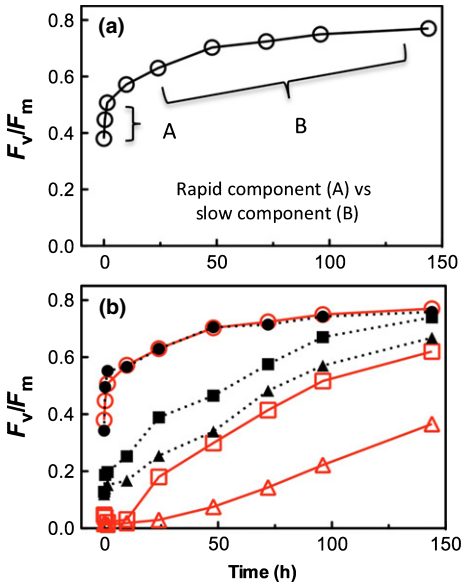

Fig. 1 Recovery kinetics of photochemical efficiency of photosystem II (F_v/F_m). (a) The two components of recovery. (b) Time series for white pine (red, solid line, open symbols) and balsam fir (black, dashed line, closed symbols): 29 November 2007 (circles), 1 January 2008 (squares), 28 February 2008 (triangles). Time zero was measured in the field after 3 h of dark acclimation. The remaining measurements were done on needles maintained in a laboratory at room temperature and in low light. Data are from Verhoeven (2013).

Species variation in the kinetics of recovery from winter stress

Studies examining multiple species demonstrate that species vary in the kinetics of recovery from winter stress upon warming (Monson *et al.*, 2005; Verhoeven *et al.*, 2009; Verhoeven, 2013). A study of conifers in a subalpine forest in Colorado showed that lodgepole pine (*Pinus contorta*) recovers more slowly than subalpine fir (*Abies lasiocarpa*) upon experimental warming in the laboratory (Monson *et al.*, 2005). Studies of conifers growing in Minnesota have shown that eastern white pine (*Pinus strobus*) recovers significantly more slowly than balsam fir (*Abies balsamea*) in the field during spring warming (Verhoeven *et al.*, 2009) as well as upon experimental warming in the laboratory (Fig. 1b, Verhoeven, 2013). Species-specific differences in recovery rate can be marked; for example, the half recovery times in February for white pine, balsam fir and white spruce (*Picea glauca*) were 144, 76 and 40 h, respectively (Verhoeven, 2013). It has been shown that temperature is the main factor constraining spring recovery of photosynthesis (e.g. Ensminger *et al.*, 2004; Thum *et al.*, 2009). There are currently limited data comparing conifer species in terms of recovery rate upon spring warming. Further comparative studies are needed as differences in recovery rate may have broader ecological implications with regard to competitive interactions between species as well as forest productivity in a changing climate.

In addition to the overall rate of recovery, the kinetics of recovery have been shown to change in a given species over the course of the winter season (e.g. Fig. 1b). A study examining recovery kinetics in four conifers growing in Minnesota found that in all species observed there was a significant rapid component to recovery early

in the winter season (late November), which disappeared later in the season in needles exposed to full sun but was maintained, to some extent, in needles growing in shaded environments (Verhoeven, 2013). Additionally the half recovery time increased significantly from January to February in three of the four conifer species examined, suggesting that, in some conifers, changes in the winter conformation of the photosynthetic apparatus continue to occur throughout the winter season.

The rapidly reversible component of sustained energy dissipation The component of sustained dissipation that reverses rapidly upon warming has been reported in a limited number of field observations (Verhoeven *et al.*, 1998, 2009; Verhoeven, 2013). The rapid component is only present in conditions when temperatures are below 0°C, suggesting that subzero temperatures may directly engage this form of sustained energy dissipation. This component has been observed in shade leaves in a higher proportion than in sun leaves (Verhoeven *et al.*, 1998, 2009; Verhoeven, 2013), and has been shown to occur early in the winter season in a higher proportion than later in the winter as mentioned previously. The rapidly reversible component of sustained dissipation seems to represent a flexible mechanism for retaining energy dissipation on subzero nights when low temperatures will preclude photosynthesis the following morning but which does not engage on warmer nights when photosynthetic activity may be possible upon sunrise.

Because of its rapid reversal upon warming, the mechanism for the rapidly reversible component has been suggested to involve low-temperature maintenance of a transmembrane ΔpH, which relaxes quickly upon warming in darkness (e.g. Gilmore, 1997). More recently it has been suggested that this form of sustained energy dissipation may result from desiccation associated with low-temperature-induced extracellular freezing of water within the needle tissue, resulting in dehydration within the protoplast, with a rapid recovery upon warming the needles resulting from rehydration associated with the needles thawing (Verhoeven, 2013). This hypothesis draws upon studies of desiccation-tolerant plants, which have reported that desiccation induces large reductions in F_v/F_m that correlate with dark-retention of A + Z, and that, upon rehydration, F_v/F_m can recover extremely rapidly (timescale of minutes to hours, Proctor, 2001, 2010; Fernández-Marín *et al.*, 2009; reviewed in Garcia-Plazaola *et al.*, 2012). Dehydration-induced reductions in F_v/F_m have been shown to involve the xanthophyll pigments Z + A, which can be formed in darkness during desiccation (Fernández-Marín *et al.*, 2009). The magnitudes of the observed reductions in F_v/F_m in desiccation-tolerant plants are similar to those observed in winter-stressed evergreens, and the rate of recovery of F_v/F_m upon rehydration is similar to that of the rapid component of recovery in winter-stressed evergreens (e.g. Verhoeven *et al.*, 1998).

The slowly reversible component of sustained energy dissipation The component of sustained energy dissipation that reverses slowly upon warming has been observed in the field in multiple species (Ottander & Öquist, 1991; Ottander *et al.*, 1995; Gilmore & Ball, 2000; Öquist & Huner, 2003; Ensminger *et al.*, 2004, 2006; Verhoeven *et al.*, 2009). This form of sustained energy dissipation is retained on winter days when temperatures rise above 0°C and is the primary component of sustained energy dissipation in sun leaves/needles, but has also been observed in shade leaves/needles (Verhoeven *et al.*, 2005, 2009; Zarter *et al.*, 2006a; Verhoeven, 2013). This component of sustained energy dissipation has been suggested to involve protein reorganization such that the xanthophyll cycle is retained in a conformation for energy dissipation in the absence of a transthylakoid ΔpH (e.g. Öquist & Huner, 2003; Adams *et al.*, 2004).

Several studies have examined changes in the abundance of different photosynthetic proteins in needles or leaves collected in winter compared with summer conditions from field samples (Ottander *et al.*, 1995; Ensminger *et al.*, 2004; Zarter *et al.*, 2006a,b; Verhoeven *et al.*, 2009) or from plants acclimated to 'late autumn' vs 'summer' conditions in the growth chamber (Savitch *et al.*, 2002, 2010; Busch *et al.*, 2007, 2008), in order to gain some understanding of the protein reorganization that occurs during the transition from summer to winter conditions. Table 3 depicts a compilation of the protein changes that have been reported in eight of these studies.

In almost all cases, there is a pronounced decrease in abundance of the PSII protein D1 during winter. The most pronounced decreases were observed in field conditions, and the decrease did not vary as a function of growth light intensity, with both Zarter *et al.* (2006a) and Verhoeven *et al.* (2009) reporting similar declines in D1 abundance in samples collected in sun vs shade. Studies by Busch *et al.* (2007, 2008), examining the effects of reductions in temperature vs photoperiod on photosynthetic proteins during acclimation to winter conditions, indicated that D1 reductions occur in response to both stimuli, suggesting that reductions in D1 abundance occur as a function of the process of cold hardening that occurs during winter acclimation and are independent of excitation pressure.

Photosystem I reaction center protein abundance has only been measured in one field study, in which the abundance of the protein measured (PsaD) decreased in sun needles of both white pine and balsam fir but did not change in fir shade needles (Table 3). Reports from chamber studies indicate no change in PsaD upon acclimation of needles of lodgepole pine at 250 μmol , and 50% decreases in PsaA/B in jack pine grown at 350 or 500 μmol. Additionally PSI values were shown to decrease in response to lowered temperature but not in response to shortened photoperiod during winter acclimation (Busch *et al.*, 2007, 2008). The data suggest that, in contrast to PSII, PSI abundances have the potential to be maintained at high values during winter, but may also be reduced in response to high excitation pressure. A study measuring photosynthetic electron transport in Scots pine demonstrated that PSI photochemistry is less inhibited during winter relative to PSII photochemistry (Ivanov *et al.*, 2001).

Studies examining changes in individual light-harvesting proteins show variable responses to winter conditions (Table 3). Overall, the data suggest that adjustments in the abundance of individual light-harvesting proteins during winter acclimation may vary as a function of growth light and temperature environment, and may differ between species. Few studies have examined all of

Table 3 Compilation of protein data in response to winter conditions, with arrows indicating decreases (↓), no change (–) or increases (↑) in the abundance of protein in winter compared with summer, and with percentage change in parentheses unless the change was not quantified (nq)

		PSII proteins		PSI proteins		Other	
Reference, species and location[1]	Growth conditions	RC (D1)	Lhcb proteins (Lhcb1–6)	RC[2]	Lhca proteins (Lhca1–4)	PsbS	ELIP
Ottander *et al.* (1995) Scots pine, Sweden	Sun	↓ (10%)	↓ (LHCII) (*c.* 45%)			↑ nq	
Ensminger *et al.* (2004)Scots pine, Central Siberia	Sun	↓ nq	–, Lhcb1 and 4 nq		–, Lhca1 nq	↓ nq	↑ nq
Zarter *et al.* (2006a,b) *Arctostaphylos uva-ursi*, CO, USA (low and high altitudes, sun and shade)	High altitude, sun/shade	↓ (15%) both				–, ↑ sun, shade	↑,↑ sun, shade
	Low altitude, sun/shade	–, ↓ (80%) sun, shade				–, ↑ sun, shade	↑,↑ sun, shade
Verhoeven *et al.* (2009) Eastern white pine and balsam fir (sun and shade), MN, USA	Pine and fir, sun	↓ (50%)	↓ (Lhcb1,4 *c.* 10%) ↓ (Lhcb2,5 *c.* 50%)	↓ (10%)	↓ (Lhca1,2, *c.* 10%) ↓ (Lhca4, *c.* 25%)	↓ (50%)	
	Fir, shade	↓ (50%)	–, (Lhcb1,2)	–	Shade: –, (Lhca1,2,4)	–	
Savitch *et al.* (2002) Lodgepole pine, chamber	250 μmol	↓ nq	↓ (Lhcb1,5) nq	– nq	–, (Lhca4) nq	↑ nq	
Busch *et al.* (2007) Jack pine, chamber	350 μmol	↓ (55%)	–, (Lhcb1,2,5)	↓ (55%)	–, (Lhca1,2,4)	↓ (66%)	
Busch *et al.* (2008) Jack pine, chamber	500 μmol	↓ (20%)	↑, (Lhcb1 *c.* 70%)	↓ (50%)			
Savitch *et al.* (2010) Lodgepole pine, chamber	250 μmol	↓ nq	↓ (Lhcb1,2,4,5 nq) ↑, (Lhcb3,6 nq)	– nq	↓ (Lhca1,2,3, nq)	↑ nq	↑ nq

RC, reaction center; PsbS, photosystem II (PSII) subunit S; ELIP, early light-induced protein.
[1]In the Ensminger *et al.* (2004) study, there were additional decreases in several proteins in early or late spring, which are not depicted. For the chamber studies, Savitch *et al.* (2002, 2010) used conditions of 25 : 15°C, day : night and 17 h photoperiod at 250 μmol for summer and 5 : 5°C, 8 h photoperiod for winter conditions. For Busch *et al.* (2007, 2008) the summer conditions were 22 : 28°C, 16 h and the winter conditions were 7 : 5°C, 8 h with a light intensity of 240 μmol (2007) or 500 μmol (2008).
[2]For the PSI reaction center, the protein monitored was either PsaD (Savitch *et al.*, 2002, 2010; Verhoeven *et al.*, 2009) or PsaA/B (Busch *et al.*, 2007, 2008) .

the individual light-harvesting proteins, so there are currently few data to determine whether individual light-harvesting proteins are enriched in winter conditions.

Other proteins of interest include the early light-induced protein (ELIP) and the PsbS protein (Table 3). ELIPs are members of a family of stress proteins that are related to LHCs and are located in the thylakoid membranes of plants (Adamska, 2001). The abundance of ELIP proteins increased during winter in all studies in which it was monitored. Changes in the PsbS protein were more variable. Comparisons of plants growing in sun vs shade during winter have shown pronounced light effects, with shade plants of *Arctostaphylos uva-ursi* showing significant increases in PsbS during winter while sun plants showed no change in PsbS, and shade fir showing no change in this protein in winter while sun fir showed significant decreases.

Although studies examining changes in the abundance of photosynthetic proteins have not directly probed the mechanisms of sustained thermal dissipation, aspects of the molecular mechanism have been discussed in this context. Studies examining individual LHCs have demonstrated that once Z is bound to the LHC, the proton gradient is no longer necessary for maintaining thermal dissipation (e.g. Dall'Osto *et al.*, 2005), suggesting that sustained forms of thermal dissipation can persist in darkness if Z is maintained. The process of sustained dissipation has also been suggested to involve the sustained aggregation of light-harvesting proteins (Ensminger *et al.*, 2006; Johnson *et al.*, 2011; Horton, 2012; Ruban *et al.*, 2012), with some studies examining winter-stressed conifers reporting increased winter aggregation of LHCs (Ottander *et al.*, 1995; Busch *et al.*, 2007). Additionally, a role for the maintained phosphorylation of the D1 protein during winter has been suggested (Ebbert *et al.*, 2005).

Synthesis of winter observations with studies on the mechanism(s) of NPQ

The component of sustained energy dissipation that reverses rapidly upon warming is similar to qE in its relaxation dynamics, as qE relaxes within seconds to minutes upon darkening, while the rapid component of sustained energy dissipation relaxes within minutes upon warming (Fig. 2). It is possible that the rapidly reversible component is a sustained form of the Q1 type of quenching (Holzwarth *et al.*, 2009), which has been shown to

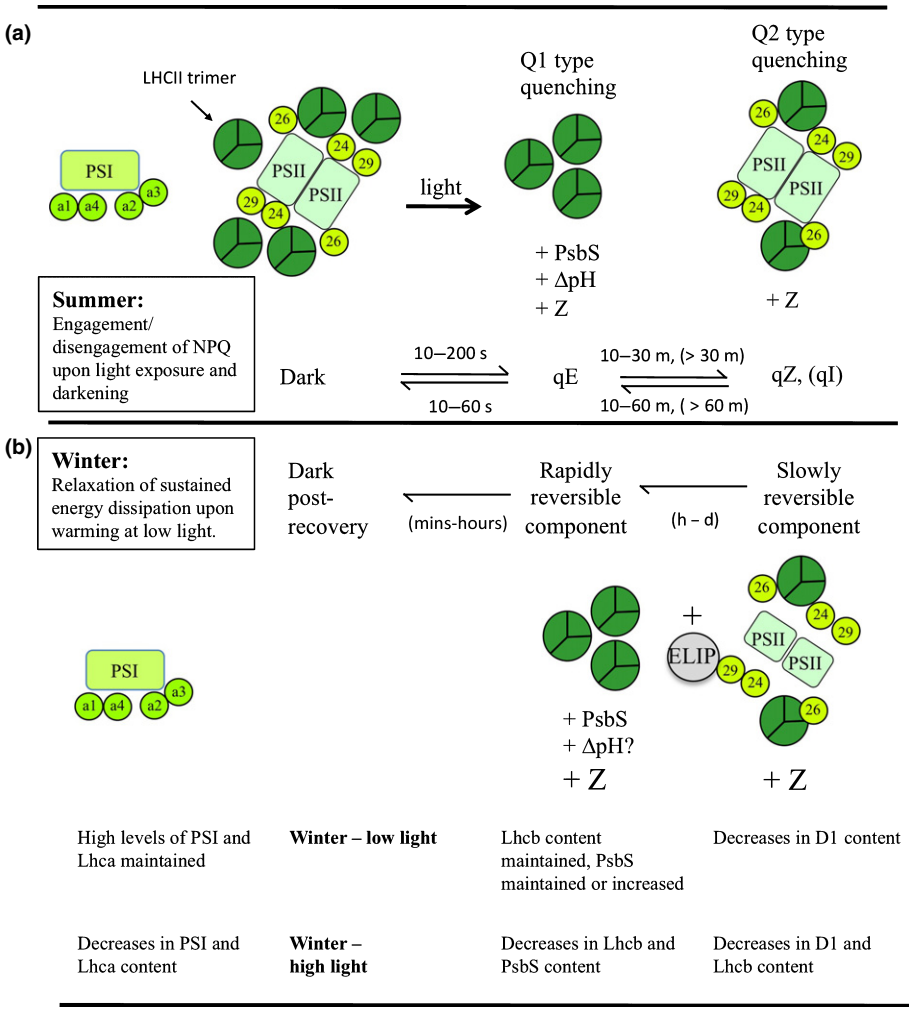

Fig. 2 (a) A schematic view of photosystem organization in unstressed conditions (according to Dekker & Boekema, 2005) and quenching locations in high light (according to Holzwarth *et al.*, 2009). The major light-harvesting antennae are shown in darker green. The minor antennae associated with photosystem II (PSII) (CP24, CP26, and CP29) are indicated by their respective numbers, while PSI antenna (Lhcba1–4) are indicated as a1–a4. Additionally the times for engagement and disengagement of different forms of nonphotochemical quenching of Chl fluorescence (NPQ) in unstressed conditions are included (Holzwarth *et al.*, 2009). (b) A proposed schematic in winter conditions illustrating the time-frame for the relaxation kinetics (upon warming) of the two forms of sustained thermal dissipation that occur under subzero conditions during winter. It is proposed that the rapidly reversible component is a sustained form of the Q1 type of quenching and the slowly reversible component is a sustained form of the Q2 type of quenching, as indicated. Observed changes in protein content in winter conditions in low vs high light conditions are indicated. PsbS, PSII subunit S; Z, zeaxanthin; ELIP, early light-induced protein; qE, rapidly reversible component of energy dissipation; qZ, slowly reversible component of energy dissipation; qI, very slowly reversible component of energy dissipation; ΔpH, proton gradient across the thylakoid membrane.

involve aggregation of the light-harvesting complex trimers (Lhcb1-3) and which requires a ΔpH, PsbS and Z (Jahns & Holzwarth, 2012; Ruban *et al.*, 2012). This is consistent with the observation that this type of quenching is observed more frequently in winter-stressed needles in the shade, which maintain higher amounts of LHCs and of PsbS than those in the sun (Zarter *et al.*, 2006a; Verhoeven *et al.*, 2009; Verhoeven, 2013). The requirement for subzero temperatures to engage this form of energy dissipation, and its very rapid reversal upon warming, suggest that this form of energy dissipation may involve a mechanism that is similar to that of the desiccation-tolerant plants in which sustained energy dissipation engages and disengages as a function of desiccation and rehydration. Further research is needed to explore the prevalence of this form of sustained energy dissipation as well as the mechanism(s) involved.

The component of sustained energy dissipation that reverses slowly is denoted by the qI (and possibly qZ) types of NPQ based on its slow reversal upon warming. This form of sustained energy dissipation may involve a sustained form of the Q2 type of quenching that has been shown to be dependent upon Z and located in the minor antenna of PSII, and to be independent of ΔpH and of PsbS (Holzwarth *et al.*, 2009; Jahns & Holzwarth, 2012). This is also somewhat consistent with field data, which show that in sun needles, in which the slowly reversible form of energy dissipation is prevalent, there are lower abundances of PsbS and light-harvesting proteins, and very high retained Z (Ottander *et al.*, 1995; Verhoeven *et al.*, 2009). Further studies examining the protein complement of winter-stressed evergreens, with high levels of slowly reversible sustained energy dissipation, are needed in order to provide an insight into the protein complement of this quenching complex.

Conclusions

A Tansley review on the topic of photoprotection was published in this journal in 2006 (Demmig-Adams & Adams, 2006). Since that time, progress has been made in our understanding of the mechanisms of NPQ in nonstressful conditions (reviewed in Jahns & Holzwarth, 2012; Ruban *et al.*, 2012), allowing for new insights into the sustained forms of energy dissipation observed during winter. Additionally we have an improved understanding of the protein changes that occur upon acclimation to winter conditions, a greater understanding of the dynamics of the two forms of sustained dissipation, as well as a recognition that species differ in the kinetics of recovery from winter stress upon warming.

A review of the data suggests that two forms of sustained energy dissipation occur in winter-stressed evergreens, and that both the light environment and the severity of the winter conditions play a role in determining the relative amount of each type of sustained energy dissipation throughout the winter (Fig. 2). A general response to the onset of winter is the down-regulation of carbon fixation, reductions in abundance of the D1 protein, and increases in ELIPs.

In lower light conditions the complement of PSI and its light-harvesting antenna seem to be maintained at values similar to those in summer. In such conditions, the PSII light-harvesting antennae are also maintained at relatively high values, while the PsbS protein is either maintained or increased in abundance. In low light conditions, both the rapidly and slowly reversible forms of sustained energy dissipation occur. It seems likely that this represents both forms of quenching (as described by Jahns & Holzwarth, 2012), with the Q1 type decreasing with the progression of the winter season. Maintenance of both forms of sustained energy dissipation provides winter-stressed needles with a high degree of photoprotection while allowing some flexibility to decrease energy dissipation on warmer days, potentially allowing for some carbon fixation.

In high light conditions, evergreens maintain a relatively low abundance of reaction center proteins for both PSII and PSI, and a reduced complement of light-harvesting proteins and PsbS, but with increased abundance of ELIP proteins. In these conditions, the rapidly reversible form of sustained energy dissipation has been observed early in the season, but is replaced by the slowly reversible form of sustained energy dissipation as the season progresses. This seems likely to represent the Q1 type of quenching being replaced by the Q2 type of quenching. Maintenance of this conformation of the photosynthetic apparatus during winter affords the needles a high degree of photoprotection, but a loss of flexibility in responding to warmer periods during winter when some carbon gain might be possible.

Future studies aimed at understanding the mechanisms of both the rapidly reversible and the slowly reversible forms of sustained energy dissipation are needed and will contribute to an improved understanding of the mechanisms of NPQ in general. Additionally, further studies examining species-specific differences in the timing of the engagement and disengagement of sustained forms of energy dissipation during acclimation to, and recovery from, winter stress will contribute to better predictions of forest responses to climate change.

References

Adams WW III, Demmig-Adams B. 1994. Carotenoid composition and down regulation of photosystem II in three conifer species during the winter. *Physiologia Plantarum* **92**: 451–458.

Adams WW III, Zarter CR, Ebbert V, Demmig-Adams B. 2004. Photoprotective strategies of overwintering evergreens. *BioScience* **54**: 41–50.

Adamska I. 2001. The ELIP family of stress proteins in the thylakoid membranes of pro and eukaryote. In: Aro EM, Andersson B, eds. *Advances in photosynthesis and respiration – regulation of photosynthesis*. Dordrecht, the Netherlands: Kluwer, 487–505.

Bigras FJ, Ryyppo A, Linsgrom A, Stattin E. 2001. Cold acclimation and deacclimation of shoots and roots of conifer seedlings. In: Bigras FJ, Colombo SJ, eds. *Conifer cold hardiness*. London, UK: Kluwer, 57–88.

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*. *Photosynthesis Research* **25**: 173–185.

Busch F, Huner NPA, Ensminger I. 2007. Increased air temperature during simulated autumn conditions does not increase photosynthetic carbon gain but affects the dissipation of excess energy in seedlings of the evergreen conifer Jack pine. *Plant Physiology* **143**: 1242–1251.

Busch F, Huner NPA, Ensminger I. 2008. Increased air temperature during simulated autumn conditions impairs photosynthetic electron transport between photosystem II and photosystem I. *Plant Physiology* **147**: 402–414.

Dall'Osto L, Caffarri S, Bassi R. 2005. A mechanism of nonphotochemical energy dissipation, independent from PsbS, revealed by a conformational change in the antenna protein CP26. *Plant Cell* **17**: 1217–1232.

Dekker JP, Boekema EJ. 2005. Supramolecular organization of thylakoid membrane proteins in green plants. *Biochimica et Biophysica Acta* **1706**: 12–39.

Demmig-Adams B, Adams WW III. 2006. Photoprotection in an ecological context: the remarkable complexity of thermal energy dissipation. *New Phytologist* **172**: 11–21.

Demmig-Adams B, Cohu CM, Muller O, Adams WW III. 2012. Modulation of photosynthetic energy conversion efficiency in nature: from seconds to seasons. *Photosynthesis Research* **113**: 75–88.

Ebbert V, Adams WW III, Mattoo AK, Sokolenko A, Demmig-Adams B. 2005. Up-regulation of a photosystem II core protein phosphatase inhibitor and sustained D1 phosphorylation in zeaxanthin-retaining, photoinhibited needles of overwintering Douglas fir. *Plant, Cell & Environment* **28**: 232–240.

Ensminger I, Busch F, Huner NPA. 2006. Photostasis and cold acclimation: sensing low temperature through photosynthesis. *Physiologia Plantarum* **126**: 28–44.

Ensminger I, Sveshnikov D, Campbell DA, Funk C, Jansson S, Lloyd J, Shibistova O, Öquist G. 2004. Intermittent low temperatures constrain spring recovery of photosynthesis in boreal Scots pine forests. *Global Change Biology* **10**: 995–1008.

Fernández-Marín B, Balaguer L, Esteban R, Becerril JM, García-Plazaola JI. 2009. Dark induction of the photoprotective xanthophyll cycle in response to dehydration. *Journal of Plant Physiology* **166**: 1734–1744.

Garcia-Plazaola JI, Esteban R, Fernandez-Marin B, Kranner I, Porcar-Castell A. 2012. Thermal energy dissipation and xanthophyll cycles beyond the *Arabidopsis* model. *Photosynthesis Research* **113**: 89–103.

Gilmore AM. 1997. Mechanistic aspects of xanthophyll cycle-dependent photoprotection in higher plant chloroplasts and leaves. *Physiologia Plantarum* **99**: 197–209.

Gilmore AM, Ball MC. 2000. Protection and storage of chlorophyll in overwintering evergreens. *Proceedings of the National Academy of Science, USA* **97**: 11098–11101.

Holzwarth AR, Miloslavina Y, Nilkens M, Jahns P. 2009. Identification of two quenching sites active in the regulation of photosynthetic light-harvesting studied by time-resolved fluorescence. *Chemical Physics Letters* **483**: 262–267.

Horton P. 2012. Optimization of light harvesting and photoprotection: molecular mechanisms and physiological consequences. *Philosophical Transactions of the Royal Society B, Biological Sciences* **367**: 3455–3465.

Ivanov AG, Sane PV, Zeinalov Y, Malmbery G, Gardeström P, Huner NPA, Öquist G. 2001. Photosynthetic electron transport adjustments in overwintering Scots pine (*Pinus sylvestris* L.). *Planta* **231**: 575–585.

Jahns P, Holzwarth AR. 2012. The role of the xanthophyll cycle and of lutein in photoprotection of photosystem II. *Biochimica et Biophysica Acta* **1817**: 182–193.

Johnson MP, Goral TK, Duffy CDP, Brain APR, Mullineaux CW, Ruban AV. 2011. Photoprotective energy dissipation involves the reorganization of photosystem II light-harvesting complexes in the grana membranes of spinach chloroplasts. *Plant Cell* **23**: 1468–1479.

Levitt J. 1980. *Responses of plants to environmental stresses, 2nd edn*. New York, NY, USA: Academic.

Li X-P, Gilmore AM, Caffarri S, Bassi R, Golan T, Kramer D, Niyogi KK. 2004. Regulation of photosynthetic light harvesting involves intrathylakoid lumen pH sensing by the PsbS protein. *Journal of Biological Chemistry* **279**: 22866–22874.

Monson RK, Sparks JP, Rosenstiel TN, Scott-Denton LE, Huxman TE, Harley PC, Turnipseed AA, Burns SP, Backlund B, Hu J. 2005. Climatic influences on net CO_2 exchange during the transition from wintertime carbon source to springtime carbon sink in a high-elevation, subalpine forest. *Oecologia* **146**: 130–147.

Müller P, Li X-P, Niyogi KK. 2001. Non-photochemical quenching. A response to excess light energy. *Plant Physiology* **125**: 1558–1566.

Murchie EH, Niyogi KK. 2011. Manipulation of photoprotection to improve plant photosynthesis. *Plant Physiology* **155**: 86–92.

Nilkens M, Kress E, Lambrev P, Miloslavina Y, Muller M, Holzwarth AR, Jahns P. 2010. Identification of a slowly inducible zeaxanthin-dependent component of non-photochemical quenching of chlorophyll fluorescence generated under steady-state conditions in *Arabidopsis*. *Biochimica et Biophysica Acta* **1797**: 466–475.

Öquist G, Huner NPA. 2003. Photosynthesis of overwintering evergreen plants. *Annual Review of Plant Biology* **54**: 329–355.

Öquist G, Ögren E. 1985. Effects of winter stress on photosynthetic electron transport and energy distribution between the two photosystems of pine as assayed by chlorophyll fluorescence kinetics. *Photosynthesis Research* **7**: 19–30.

Ottander C, Campbell D, Öquist G. 1995. Seasonal changes in photosystem II organization and pigment composition in *Pinus sylvestris*. *Planta* **197**: 176–183.

Ottander C, Öquist G. 1991. Recovery of photosynthesis in winter-stressed Scots pine. *Plant, Cell & Environment* **14**: 345–349.

Porcar-Castell A. 2011. A high-resolution portrait of the annual dynamics of photochemical and non-photochemical quenching in needles of *Pinus sylvestris*. *Physiologia Plantarum* **143**: 139–153.

Proctor MCF. 2001. Patterns of desiccation tolerance and recovery in bryophytes. *Plant Growth Regulation* **35**: 147–156.

Proctor MCF. 2010. Recovery rates of chlorophyll-fluorescence parameters in desiccation-tolerant plants: fitted logistic curves as a versatile and robust source of comparative data. *Plant Growth Regulation* **62**: 233–240.

Ruban AV, Johnson MP, Duffy CDP. 2012. The photoprotective molecular switch in the photosystem II antenna. *Biochimica et Biophysica Acta* **1817**: 167–181.

Savitch LV, Ivanov A, Krol M, Sprott DP, Oquist G, Huner NPA. 2010. Regulation of energy partitioning and alternative electron transport pathways during cold acclimation of lodgepole pine is oxygen dependent. *Plant Cell Physiology* **51**: 1555–1570.

Savitch LV, Leonardos ED, Krol M, Jansson S, Grodzinski B, Huner NPA, Öquist G. 2002. Two different strategies for light utilization in photosynthesis in relation to growth and cold acclimation. *Plant, Cell & Environment* **25**: 761–771.

Takahashi S, Badger MR. 2011. Photoprotection in plants: a new light on photosystem II damage. *Trends in Plant Science* **16**: 53–60.

Thum T, Aalto T, Laurila T, Aurela M, Hatakka J, Lindroth A. 2009. Spring initiation and autumn cessation of boreal coniferous forest CO_2 exchange assessed by meteorological and biological variables. *Tellus* **61B**: 701–717.

Verhoeven A, Osmolak A, Morales P, Crow J. 2009. Seasonal changes in abundance and phosphorylation status of photosynthetic proteins in eastern white pine and balsam fir. *Tree Physiology* **29**: 362–374.

Verhoeven A, Swanberg A, Thao M, Whiteman J. 2005. Seasonal changes in leaf antioxidant systems and xanthophyll cycle characteristics in *Taxus x media* growing in sun and shade environments. *Physiologia Plantarum* **123**: 428–434.

Verhoeven AS. 2013. Recovery kinetics of photochemical efficiency in winter stressed conifers: the effects of growth light environment, extent of the season and species. *Physiologia Plantarum* **147**: 147–158.

Verhoeven AS, Adams WW III, Demmig-Adams B. 1996. Close relationship between the state of the xanthophyll cycle pigments and photosystem II efficiency during recovery from winter stress. *Physiologia Plantarum* **96**: 567–576.

Verhoeven AS, Adams WW III, Demmig-Adams B. 1998. Photoinhibition during winter stress in sun and shade *Euonymus kiautschovicus* leaves; the xanthophyll cycle and leaf adenylate status. *Plant, Cell & Environment* **21**: 893–903.

Zarter CR, Adams WW III, Ebbert V, Adamska I, Jansson S, Demmig-Adams B. 2006a. Winter acclimation of PsbS and related proteins in the evergreen *Arctostaphylos uva-ursi* as influenced by altitude and light environment. *Plant, Cell & Environment* **29**: 869–878.

Zarter CR, Demmig-Adams B, Ebbert B, Adamska I, Adams WW III. 2006b. Photosynthetic capacity and light harvesting efficiency during the winter to spring transition in subalpine conifers. *New Phytologist* **172**: 283–292.