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 (Oquist & 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 (LHCCI) 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

<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP24</td>
<td>Minor light-harvesting antennae protein, also called Lhcb6</td>
</tr>
<tr>
<td>CP26</td>
<td>Minor light-harvesting antennae protein, also called Lhcb5</td>
</tr>
<tr>
<td>CP29</td>
<td>Minor light-harvesting antennae protein, also called Lhcb4</td>
</tr>
<tr>
<td>ELIP</td>
<td>Early light-induced protein, family of proteins related to light-harvesting proteins that are involved in stress responses</td>
</tr>
<tr>
<td>Energy dissipation</td>
<td>A process whereby excitation energy absorbed by Chl is dissipated thermally. Commonly measured using the Chl fluorescence parameter NPQ.</td>
</tr>
<tr>
<td>$F_{v}/F_{m}$</td>
<td>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</td>
</tr>
<tr>
<td>LHC</td>
<td>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 LHCCI trimers, which are a combination of Lhcb1–3, and the minor LHC proteins, which exist as monomers (Lhcb4–6)</td>
</tr>
<tr>
<td>NPQ</td>
<td>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</td>
</tr>
<tr>
<td>qE</td>
<td>The component of NPQ that reverses rapidly (seconds to minutes) upon darkening a leaf that has been exposed to high light</td>
</tr>
<tr>
<td>qI</td>
<td>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</td>
</tr>
<tr>
<td>qZ</td>
<td>The component of NPQ that reverses between 10 and 30 min upon darkening a leaf that has been exposed to high light</td>
</tr>
<tr>
<td>Q1 quenching</td>
<td>A type of fluorescence quenching proposed by Holzwarth and colleagues that occurs within LHCCI trimers that have disassociated from the PSII core and that requires PsbS, a ΔpH and Z (zeaxanthin)</td>
</tr>
<tr>
<td>Q2 quenching</td>
<td>A type of fluorescence quenching proposed by Holzwarth and colleagues that occurs within the minor LHC antennae associated with PSI and that requires Z but can occur independently of ΔpH and PsbS</td>
</tr>
<tr>
<td>ΔpH</td>
<td>A gradient in pH across the thylakoid membrane that occurs when plants are undergoing photosynthesis</td>
</tr>
<tr>
<td>PsbS</td>
<td>A protein in the light-harvesting family of proteins that has been shown to be essential in the process of thermal energy dissipation</td>
</tr>
</tbody>
</table>
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 (Oquist & 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 (Oquist & Huner, 2003).

### Early studies examining Chl fluorescence of overwintering evergreens

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 ($\frac{A + Z}{V + A + Z}$), total amount of xanthophyll pigments ($V + A + Z$), and lutein content in summer (S) compared with winter (W)

<table>
<thead>
<tr>
<th>Species</th>
<th>AZ/(VAZ)</th>
<th>$V + A + Z$</th>
<th>Summer (%)</th>
<th>Winter (%)</th>
<th>Lutein</th>
<th>Winter (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinus sylvestris$^1$</td>
<td>0.2</td>
<td>0.025</td>
<td>100%</td>
<td>0.055</td>
<td>82%</td>
<td></td>
</tr>
<tr>
<td>Pinus ponderosa$^2$</td>
<td>0.35</td>
<td>0.223</td>
<td>23%</td>
<td>0.384</td>
<td>81%</td>
<td></td>
</tr>
<tr>
<td>Pseudotsuga menziesii$^2$</td>
<td>0.68</td>
<td>0.218</td>
<td>78%</td>
<td>0.460</td>
<td>20%</td>
<td></td>
</tr>
<tr>
<td>Picea pungens$^2$</td>
<td>0.12</td>
<td>0.128</td>
<td>112%</td>
<td>0.251</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>Euonymus kiautschovicus$^3$ (sun)</td>
<td>0.85</td>
<td>0.47</td>
<td>87%</td>
<td>0.251</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>E. kiautschovicus$^4$ (shade)</td>
<td>0.85</td>
<td>22</td>
<td>100%</td>
<td>0.174</td>
<td>61%</td>
<td></td>
</tr>
<tr>
<td>Taxus x media$^5$ (sun)</td>
<td>0.85</td>
<td>1.14</td>
<td>65%</td>
<td>0.174</td>
<td>61%</td>
<td></td>
</tr>
<tr>
<td>Taxus x media$^5$ (shade)</td>
<td>0.75</td>
<td>49</td>
<td>49%</td>
<td>0.124</td>
<td>51%</td>
<td></td>
</tr>
<tr>
<td>Abies balsamea$^6$ (sun)</td>
<td>0.60</td>
<td>86</td>
<td>123%</td>
<td>0.144</td>
<td>118%</td>
<td></td>
</tr>
<tr>
<td>A. balsamea$^6$ (shade)</td>
<td>0.09</td>
<td>55</td>
<td>30%</td>
<td>0.135</td>
<td>58%</td>
<td></td>
</tr>
<tr>
<td>Pinus strobus$^5$</td>
<td>0.39</td>
<td>82</td>
<td>63%</td>
<td>0.148</td>
<td>90%</td>
<td></td>
</tr>
</tbody>
</table>

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)$^{-1}$.

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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 for 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, Otter N & Øquist, 1991; Ottander & Alström, 1995; Verhoeven et al., 1996, 1998, 2009; Verhoeven, 2013). Both phases correlate with the conversion 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.

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 García-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 (PsdA) 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 PsdA upon acclimation of needles of lodgepole pine at 250 μmol and 50% decreases in PsA/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
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 LHCs, which has been shown to synthesize 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...
involves 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.

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.

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 (Lhcb1-4) 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.
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 configuration 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


Research review

A high-resolution portrait of the annual dynamics of Porcar-Castell A. 2011.


