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HIGHLIGHTED STUDENT RESEARCH

Wide variation of winter-induced sustained thermal energy dissipation in conifers: a common-garden study

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Abstract

Low temperature in winter depresses rates of photosynthesis, which, in evergreen plants, can exacerbate imbalances between light absorption and photochemical light use. Damage that could result from increased excess light absorption is minimized by the conversion of excitation energy to heat in a process known as energy dissipation, which involves the de-epoxidized carotenoids of the xanthophyll cycle. Overwintering evergreens employ sustained forms of energy dissipation observable even after lengthy periods of dark acclimation. Whereas most studies of photoprotective energy dissipation examine one or a small number of species; here, we measured the levels of sustained thermal energy dissipation of seventy conifer taxa growing outdoors under common-garden conditions at the Red Butte Garden in Salt Lake City, Utah, U.S.A. (forty nine taxa were also sampled for needle pigment content). We observed an extremely wide range of wintertime engagement of sustained energy dissipation; the percentage decrease in dark-acclimated photosystem II quantum efficiency from summer to winter ranged from 6 to 95%. Of the many pigment-based parameters measured, the magnitude of the seasonal decrease in quantum efficiency was most closely associated with the seasonal increase in zeaxanthin content expressed on a total chlorophyll basis, which explained only slightly more than one-third of the variation. We did not find evidence for a consistent wintertime decrease in needle chlorophyll content. Thus, the prevailing mechanism for winter decreases in solar-induced fluorescence emitted by evergreen forests may be decreases in fluorescence quantum yield, and wintertime deployment of sustained energy dissipation likely underlies this effect.

Keywords Chlorophyll fluorescence · Needle chlorophylls and carotenoids · Xanthophyll cycle · Winter dormancy · Zeaxanthin

Introduction

Winter can be a period of great stress for evergreen conifers. Frozen soils and stems limit water availability. Ice formation can damage macromolecules, cells, tissues and organs (reviewed in Hochachka and Somero 1984; Steponkus 1984; Sakai and Larcher 1987), and snow accumulation on branches retaining their foliage can result in mechanical stress and loss of limbs (Tranquillini 2012; Sakai and Larcher 1987; Niklas 1991). Winter brings considerable metabolic challenges, as well. Low temperature inhibits enzyme activities, slowing the reactions that lead to photosynthetic carbon assimilation and growth. Histological analyses of high-altitude conifers (Rossi et al. 2007) and growth experiments with evergreen seedlings (Alvarez-Uria and Körner 2007) conclude that xylem cell differentiation and root extension both cease entirely below 5 °C. Seasonal

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In this first-of-its-kind common-garden study, we report astonishing variation among seventy conifer taxa in their winter engagement of sustained energy dissipation, a key photoprotective mechanism.

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downregulation of photosynthetic capacity further reduces carbon assimilation in winter (Öquist and Huner 2003).

Although low temperature inhibits the enzyme-catalyzed reactions of photosynthesis, it has little impact on light absorption (Verhoeven 2014). This can exacerbate the imbalance between the amount of energy absorbed by chlorophyll in the light-harvesting complexes and its utilization to support photochemistry (Öquist and Huner 2003). Such imbalances can lead to the formation of reactive oxygen species (Krieger-Liszkay 2005; Foyer et al. 1994), which can damage cellular macromolecules and lead to leaf mortality (Ledford and Niyogi 2005). Overwintering conifers are thus especially vulnerable to stress associated with excess light absorption.

Photosynthetic organisms have evolved a host of photoprotective strategies that minimize the deleterious effects of excess light absorption (reviewed in Logan et al. 1999; Logan 2006). One such strategy, ubiquitous among higher plants, is thermal energy dissipation: the conversion of light energy absorbed by chlorophyll to heat (Demmig-Adams and Adams 1992, 2006; Esteban et al. 2015). This process limits the formation of reactive oxygen species and is facilitated by the de-epoxidized carotenoids of the xanthophyll cycle, zeaxanthin (Z) and possibly antheraxanthin (A), which accumulate when energy dissipation is engaged (Demmig-Adams and Adams 1992). During the growing season, levels of energy dissipation respond to varying light intensity over time scales of seconds to minutes, in part, by interconversions between Z and A and violaxanthin (V), the epoxidized member of the xanthophyll cycle (V + A + Z), which does not facilitate energy dissipation. Conversion of V to A and Z is catalyzed by violaxanthin de-epoxidase. Over time scales of days to seasons, the capacity for thermal energy dissipation acclimates to growth conditions through adjustments in the size of the xanthophyll cycle pool of pigments (Logan et al. 1996, 1998). These adjustments ensure that excess light damage is minimized, while also limiting the dissipation of absorbed light that could otherwise have led to photosynthetic carbon assimilation (Kromdijk et al. 2016). Zeaxanthin has also been shown to limit lipid peroxidation in the thylakoid membrane (Jahns et al. 2012).

The dynamic form of thermal energy dissipation described above generally prevails during the growing season. However, just as the enzyme-catalyzed reactions of photosynthesis and growth are inhibited by low temperature, so too is violaxanthin de-epoxidase-mediated conversion of V to Z. Thus, evergreens employ sustained energy dissipation to cope with the cold of winter (Adams et al. 2004; Verhoeven et al. 2014). Sustained energy dissipation involves changes in photosystem II (PSII)-protein expression and conformation, an increase in the size of the xanthophyll cycle pool, and the retention of Z and A (Demmig-Adams and Adams 2006; Verhoeven et al. 2014). Decreased PSII quantum yield, measurable as a decrease in the chlorophyll fluorescence parameter F_v/F_m after a period of dark-acclimation, is a hallmark of sustained energy dissipation that is coupled with retained high xanthophyll cycle conversion states ([Z + A]/[V + A + Z]; reviewed in Adams et al. 2004). Sustained thermal energy dissipation appears to involve two components with different relaxation kinetics: a more rapidly reversible component that relaxes in minutes to hours, and a slowly reversible component that can take as many as six days of favorable conditions to disengage (Demmig-Adams and Adams 2006; Verhoeven et al. 2014). This latter component limits the possibility of premature disengagement that might expose evergreen foliage to excess-light damage after transient mid-winter warming. A decrease in chlorophyll pool size has also been documented in some, but not all, conifers as an alternative means of limiting excess light stress in winter by lowering light absorption (Öquist and Huner 2003; Ensminger et al. 2004; Wong and Gamon 2015a, b; Sofronova et al. 2016, 2018).

The relationship between sustained energy dissipation and winter photosynthetic dormancy serves as the basis for proximal- and remote-sensing methods used to characterize photosynthetic phenology. Seasonal variation in the "photochemical reflectance index" (PRI; Wong and Gamon 2015a and 2015b) and the "chlorophyll/carotenoid index" (CCI; Gamon et al. 2016) is related to seasonal acclimation in the content of xanthophyll cycle carotenoids. These adjustments to pigment content also influence the seasonality of the "green chromatic coordinate" (G_{cc}) and the "green–red vegetation index" (GRVI) determined from repeat digital photography (Richardson et al. 2007; Motohka et al. 2010). Seasonality of G_{cc} and GRVI correlate with changes in needle carotenoid content, chiefly driven by acclimation of the xanthophyll cycle in evergreen conifers exhibiting no seasonal variation in needle chlorophyll content (Bowling et al. 2018; Seyednasrollah et al. 2020).

Studies of sustained energy dissipation tend to focus upon one or a small number of species, although a recent meta-analysis of evergreens in their native habitats showed that levels and threshold temperature initiating sustained energy dissipation can vary widely across taxa (Míguez et al. 2015). Here, we report a summer–winter comparison of the photosynthetic quantum yield and leaf pigment content of 70 mature evergreen needleleaf taxa growing in a mid-latitude common garden, the Red Butte Garden, in Salt Lake City, UT, USA. Four conifer families are represented in our sample of the collection, with adequate representation to allow for a comparison of members of the *Pinaceae* to members of the *Cupressaceae.*

These families have broad, and broadly overlapping, geographical distributions and are likely to exhibit physiological adaptation and acclimation in a convergent manner. Thus, we hypothesized that seasonal responses would not differ by plant family. Individual taxa in the collection range from a 'one' (most hardy) to an 'eight' (least hardy) on the United States Department of Agriculture (USDA) hardiness index (Bannister and Neuner 2001; The American Conifer Society 2020), which is based upon the mean air temperature of the coldest month above which a species is "most likely to thrive" (Fig. 1a). The greatest seasonal decreases in photosynthetic quantum yield are generally observed in plants adapted to environments experiencing the most severe winters (Demmig-Adams and Adams 2006). Thus, we hypothesized that the magnitude of sustained energy dissipation, and any associated changes in needle pigment composition, will correlate with hardiness.

Methods

Study site and sampling

Study trees were members of The Conifer Collection at the Red Butte Garden in Salt Lake City, Utah, USA (40.77° N, 111.82° W), which is administered by the University of Utah. August temperature in Salt Lake City ranges from 17.4° C to 32.5° C, while February temperature ranges from − 3.7° C to 6.2° C (National Oceanic and Atmospheric Administration, 1981–2010 normals, https://www.ncdc.noaa.gov/cdo-web/datatools/normals; Fig. S1, gray bars). The study trees were mature and growing outdoors. Measurement periods included summer (8-August-2018 to 20-August-2018) and winter (8-February-2019 to 1-March-2019). For each taxon, only a single individual was available for sampling. Needles from a full-sun exposed terminal needle cluster of current-year foliage were used for analyses. The sampled branch was photographed and tagged during summer sampling and returned to for winter sampling of a neighboring terminal needle cluster.

Seventy individuals were selected from Red Butte Garden for measurements of chlorophyll fluorescence emission. These individuals represent 42 unique species (55 are regarded as cultivars; 15 as wildtype) from 13 genera and four families, all within the order *Pinales* (Fig. 1a), and are native to a broad range of habitats around the globe.

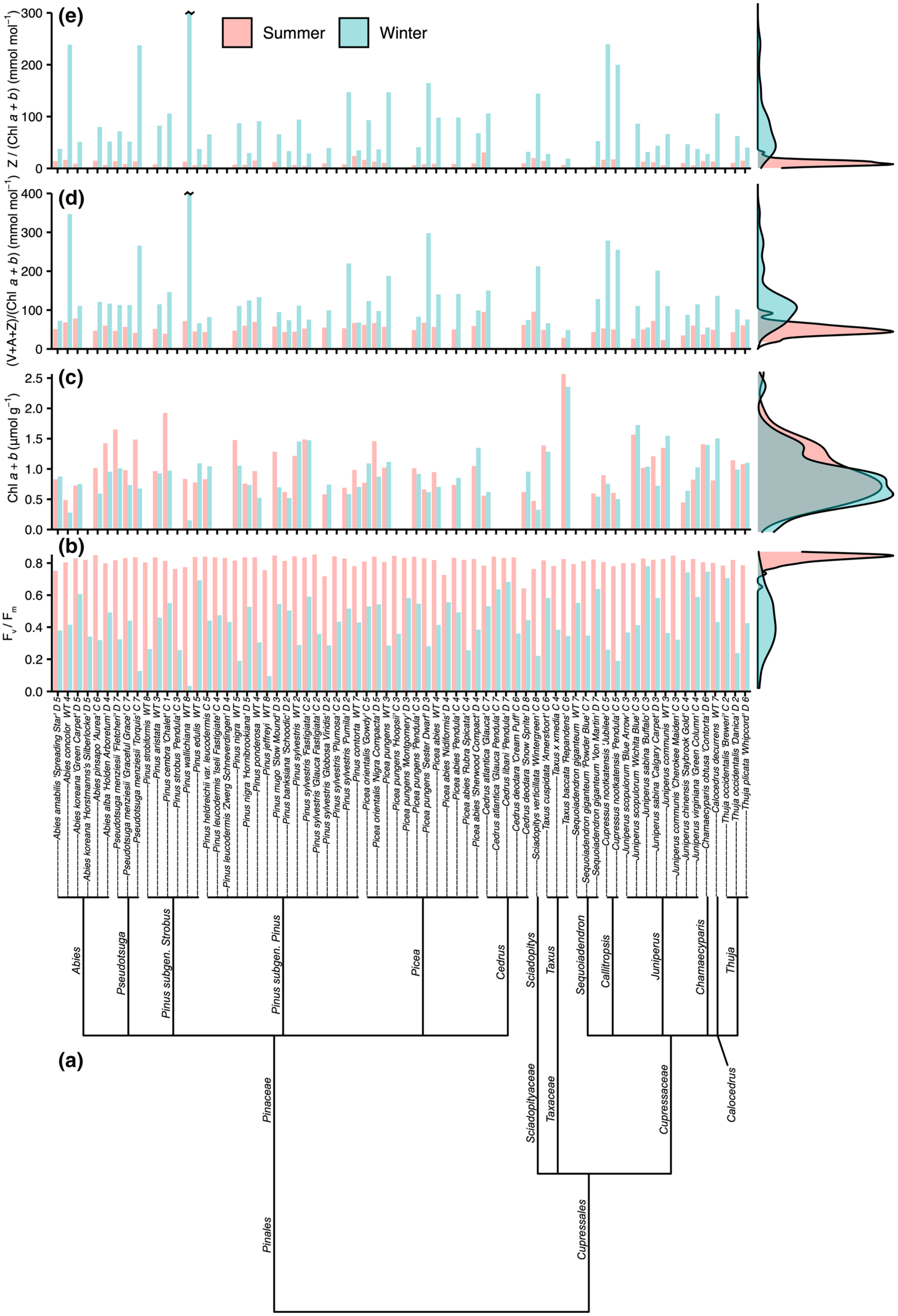
Measurements of winter and summer needle pigment content were also taken from 49 of the individuals on which analyses of chlorophyll fluorescence emission were performed. These represent 36 species drawn from all 13 genera (Fig. 1b–e). The needle pigment dataset was constructed to be taxonomically representative of the individuals from whom chlorophyll fluorescence was measured and visual inspection of the distribution of values did not indicate selection bias in Hardiness, WPI (described below) or pigment content. Our investigation into the influence of family affiliation on manifestations of sustained energy dissipation is confined to comparisons between *Pinaceae* and *Cupressaceae*, as only these two families were represented by more than three individuals in our study.

Analyses of chlorophyll fluorescence emission

Pulse-amplitude modulated (PAM) chlorophyll fluorescence emission was measured in the chamber of a temperature-controlled gas exchange system (LI-6800, LI-COR Biosciences, Lincoln, NE, USA). Target leaf temperature was 25 °C and 5 °C during summer and winter measurements, respectively {leaf temperature during measurement was 25.1 ± 0.1 in summer and 5.5 ± 0.8 in winter [mean ± standard deviation]}. Measurements were performed at 400 μmol mol^{-1} carbon dioxide and a target vapor pressure saturation deficit of 1.9 or 0.5 kPa (summer and winter, respectively). Terminal needle clusters were detached and wrapped in light-tight metal foil for a minimum 60 min period of dark acclimation (no more than 180 min) at ~ 20 °C or ~ 5 °C in summer and winter, respectively. After dark acclimation, minimal fluorescence (F_o) and maximal fluorescence in the presence of a saturating flash (F_m) were measured. Chamber light intensity was then set to 200 μmol photons m^{-2} s^{-1}. Needles equilibrated to that light intensity for five minutes, after which steady-state fluorescence emission (F_t), and maximal fluorescence emission during a saturating flash (F_m') were collected. Actinic light intensity was then set to 1600 μmol photons m^{-2} s^{-1} and F_t and F_m' were measured again after another five minutes of equilibration. Fluorescence parameters were used to determine the dark-acclimated photosystem II (PSII) quantum yield (F_v/F_m, where $F_v = F_m - F_o$) and the PSII efficiency during illumination ($\phi PSII = [F_m' - F_t]/ F_m'$; Maxwell and Johnson 2000). Depending upon needle and branch architecture, measurements were performed in the following manner: needles of sufficient length (on detached distal branches) were arranged adjacent to one another with portions enclosed in the chamber; for branches with a flat needle arrangement, portions were enclosed in the chamber; for all other taxa, needles were carefully detached from branches, positioned on adhesive tape, then enclosed in the chamber. To quantify sustained energy dissipation, we calculated an index describing the degree of winter photoinhibition (WPI) after Miguez et al. (2015):

$$\mathrm{WPI} = \frac{\mathrm{Summer}\frac{F_v}{F_m} - \mathrm{Winter}\frac{F_v}{F_m}}{\mathrm{Summer}\frac{F_v}{F_m}} \times 100,$$

The mean of measurements collected from three independent needle clusters from the same terminal branch of each of the 70 taxa examined was used for analyses. Needle tissue was then preserved for determination of needle


(e)
Summer
Winter
Z / (Chl $a+b$) (mmol mol^{-1})
300
200
100
0
(d)
(V+A+Z)/(Chl $a+b$) (mmol mol^{-1})
400
300
200
100
0
(c)
Chl $a+b$ (µmol g^{-1})
2.5
2.0
1.5
1.0
0.5
0.0
(b)
F_v / F_m
0.8
0.6
0.4
0.2
0.0
Abies amabilis 'Spreading Star' D 5
Abies concolor WT 4
Abies koreana 'Green Carpet' D 5
Abies koreana 'Horstmann's Silberlocke' D 5
Abies pinsapo 'Aurea' C 6
Abies alba 'Holden Arboretum' D 4
Pseudotsuga menziesii 'Fletcheri' D 7
Pseudotsuga menziesii 'Graceful Grace' C 7
Pseudotsuga menziesii 'Torquis' C 7
Pinus strobiformis WT 8
Pinus aristata WT 3
Pinus cembra 'Chalet' C 1
Pinus strobus 'Pendula' C 3
Pinus wallichiana WT 8
Pinus edulis WT 5
Pinus heldreichii var. leucodermis C 5
Pinus leucodermis 'Iseli Fastigiate' C 4
Pinus leucodermis 'Zwerg Schneverdingen' D 4
Pinus nigra WT 5
Pinus nigra 'Hornibrookiana' D 5
Pinus ponderosa WT 4
Pinus jeffreyi WT 8
Pinus mugo 'Slow Mound' D 3
Pinus banksiana 'Schoodic' D 2
Pinus sylvestris WT 2
Pinus sylvestris 'Fastigiata' C 2
Pinus sylvestris 'Glauca Fastigiata' C 2
Pinus sylvestris 'Globosa Viridis' D 2
Pinus sylvestris 'Plumosa' C 2
Pinus sylvestris 'Pumila' D 2
Pinus contorta WT 7
Picea orientalis 'Gowdy' C 5
Picea orientalis 'Nigra Compacta' D 5
Picea pungens WT 3
Picea pungens 'Hoopsii' C 3
Picea pungens 'Montgomery' D 3
Picea pungens 'Pendula' C 3
Picea pungens 'Sester Dwarf' D 3
Picea abies WT 4
Picea abies 'Nidiformis' D 4
Picea abies 'Pendula' C 4
Picea abies 'Rubra Spicata' C 4
Picea abies 'Sherwood Compact' D 4
Cedrus atlantica 'Glauca' C 7
Cedrus atlantica 'Glauca Pendula' C 7
Cedrus libani 'Pendula' D 7
Cedrus deodara 'Cream Puff' D 6
Cedrus deodara 'Snow Sprite' D 6
Sciadopitys verticillata 'Wintergreen' C 8
Taxus cuspidata 'Amersfoort' C 6
Taxus x media C 4
Taxus baccata 'Repandens' C 6
Sequoiadendron giganteum WT 7
Sequoiadendron giganteum 'Powder Blue' C 7
Sequoiadendron giganteum 'Von Martin' D 7
Cupressus nootkatensis 'Jubilee' C 5
Cupressus nootkatensis 'Pendula' C 5
Juniperus scopulorum 'Blue Arrow' C 3
Juniperus scopulorum 'Wichita Blue' C 3
Juniperus sabina 'Buffalo' C 3
Juniperus sabina 'Calgary Carpet' D 3
Juniperus communis WT 3
Juniperus communis 'Cherokee Maiden' C 3
Juniperus chinensis 'Saybrook Gold' C 4
Juniperus virginiana 'Green Column' C 4
Chamaecyparis obtusa 'Contorta' D 6
Calocedrus decurrens WT 7
Thuja occidentalis 'Brewer' C 2
Thuja occidentalis 'Danica' D 2
Thuja plicata 'Whipcord' D 6
Abies
Pseudotsuga
Pinus subgen. Strobus
Pinus subgen. Pinus
Picea
Cedrus
Sciadopitys
Taxus
Sequoiadendron
Callitropsis
Juniperus
Chamaecyparis
Thuja
(a)
Pinaceae
Sciadopityaceae
Taxaceae
Cupressaceae
Calocedrus
Pinales
Cupressales

◂**Fig. 1** Taxonomic relationships among study plants with genotype code (*WT* wildtype, *C* cultivar, *D* dwarf cultivar) and USDA hardiness rating (single digit numerals), (**a**) F_v/F_m (**b**), chlorophyll content on a needle fresh mass basis (**c**), xanthophyll cycle pool (V + A + Z) size and zeaxanthin (Z) content on a total chlorophyll basis (**d** and **e**, respectively). Summer observations are in pink; winter observations in blue. Smoothed frequency distributions for indicated parameters in summer and winter are depicted to the right of each panel

pigment content from a subsample of 49 taxa (as described above).

Needle pigment content

Needle pigment content (chlorophylls and carotenoids) was determined by high-performance liquid chromatography (HPLC) using a reverse-phase Carotenoid C30 column (YMC, Allentown, PA, USA) fitted to a Series 1100 HPLC with diode array detection (Agilent, Santa Clara, CA, USA) after extraction in acetone according to Bowling et al. (2018). Reported pigment data are from dark-acclimated needles. For most individuals, triplicate samples were extracted and measured (duplicate samples were used for eight individuals). Means of replicate measurements were used for analyses.

Statistical analysis

We first assessed whether temperature fluctuation over the course of winter data collection influenced comparisons of measurements collected on different days (see Table S1 for details on measurement dates for each taxon). During the period of winter measurement, minimum daily air temperature ranged from − 11.3° C to 7.3° C, and there were three measurement days during which air temperature did not fall below freezing (Fig. S1). F_v/F_m collected on these three days was not significantly different from the rest (one-way analyses of variance (ANOVA), $p = 0.98$). Furthermore, we found no correlation between winter F_v/F_m and the number of hours above 0° C nor the number of hours above 5° C on the day of winter measurement or the three days preceding winter measurement.

Pearson's correlation tests were used to explore relationships between parameters, as all parameters reported in the main text satisfied a Shapiro–Wilk test for normality ($p > 0.05$). To search for effects of taxonomic affiliation on measured quantities, ANOVAs between members of *Cupressaceae* and *Pinaceae* were performed. Relationships between WPI and hardiness or pigment content were assessed via linear regression. All statistical analyses were performed in R (R Core Team 2020), and all plots were produced with the 'ggplot2' (Wickham 2016) or 'corrplot' (Wei et al. 2017) packages. The phylogenetic tree in Fig. 1 was created in FigTree v1.4.4 (Rambaut, http://tree.bio.ed.ac.uk/software/figtree/), and all figures were finalized in Adobe Illustrator (Adobe Inc., 2019).

Results

We examined numerous parameters, including the results of chlorophyll fluorescence analyses, aspects of needle pigment content expressed on needle fresh-mass and chlorophyll bases, quantitative representations of the seasonal difference in parameters, and USDA hardiness rating. To determine which of these parameters were most meaningful in the context of sustained energy dissipation, we constructed a correlation matrix between all variables (Fig. S2).

Summer F_v/F_m was high and exhibited little variation across individuals, with only one measurement below 0.70 (*C. decurrens*; Fig. 1b), indicating that the plants in this study were generally not under chronic stress during the summer. This is consistent with the close care they received at Red Butte Garden. In winter, F_v/F_m was lower and more broadly distributed than in summer, suggesting widespread but variable engagement of sustained energy dissipation (Fig. 1b). This is also illustrated by the high variability of WPI, which ranged from 6 (*Juniperus sabina*) to 95% (*Pinus wallichiana*). Comparable distributions of winter F_v/F_m were found in both plant families, and there was no significant difference between the WPI or the winter F_v/F_m of *Pinaceae* and *Cupressaceae* (one-way ANOVA, $p = 0.12$ and 0.14, respectively).

Contrary to our hypothesis, the USDA hardiness ratings of the individuals in this study were not correlated with WPI (Fig. 2) or winter F_v/F_m. However, hardiness correlated with summer F_v/F_m and summer Z/(Chl$a + b$), among other summer pigment parameters (Fig. S2).

While the range in F_v/F_m increased greatly from summer to winter, the range in ϕPSII during illumination narrowed considerably in winter at both saturating (1600 µmol m^{-2} s^{-1}) and non-saturating (200 µmol m^{-2} s^{-1}) light intensities (Fig. 3).

Several pigment parameters associated with sustained energy dissipation, including winter Z/(V + A + Z) and winter (A + Z)/(V + A + Z) yielded coefficients of determination (r^2) of 0.31 and 0.26 when related to WPI, respectively (Fig. S2). However, the pigment variables that explained the greatest proportion of the variance in WPI were the winter increases in Z/(Chl $a + b$) and (V + A + Z)/(Chl $a + b$) ($r^2 = 0.37$ and 0.33, respectively; Fig. 4). We thus depicted these parameters, along with F_v/F_m (summer r^2 not significant, winter $r^2 = 0.98$) and total chlorophyll content (not significant) in Fig. 1. *Pinus wallichiana* stands out as possessing exceptionally large winter increases in Z/(Chl$a + b$) and (V + A + Z)/(Chl$a + b$) along with particularly high WPI. Excluding *P. wallichiana* decreased r^2 values for the

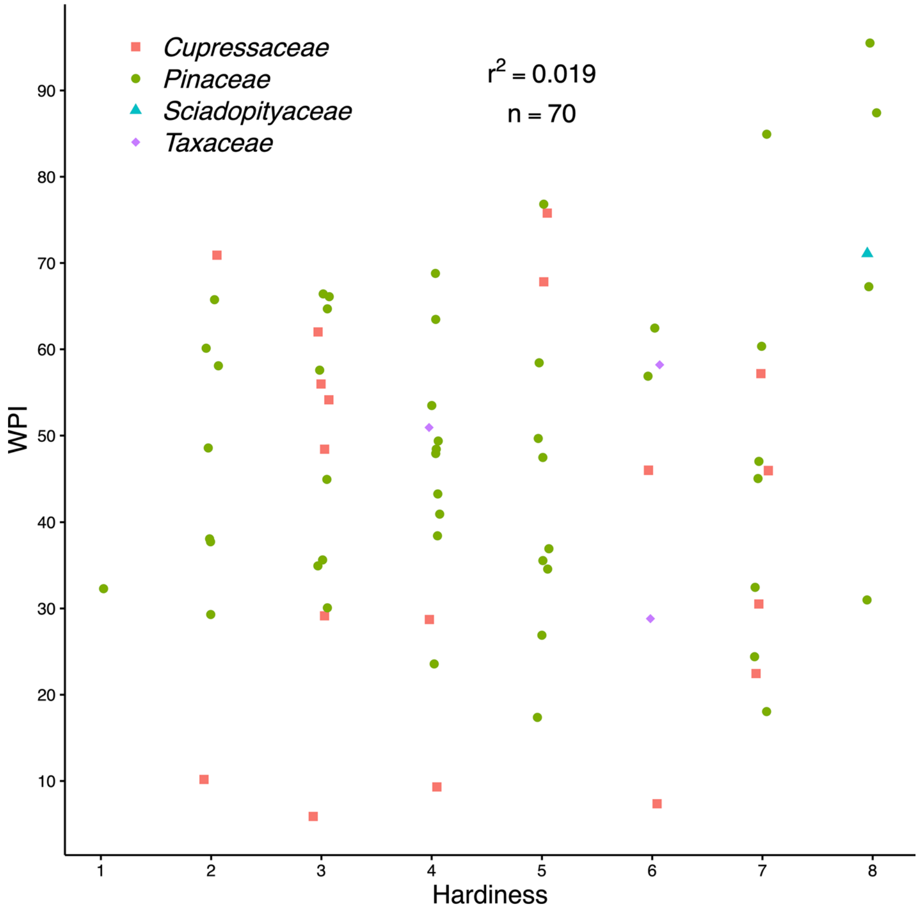


Fig. 2 Winter photoinhibition (WPI) plotted against USDA hardiness rating. Red squares = members of the *Cupressaceae*, green circles = members of the *Pinaceae*, blue triangles = a member of the *Sciadopityaceae*, purple diamonds = members of the *Taxaceae*

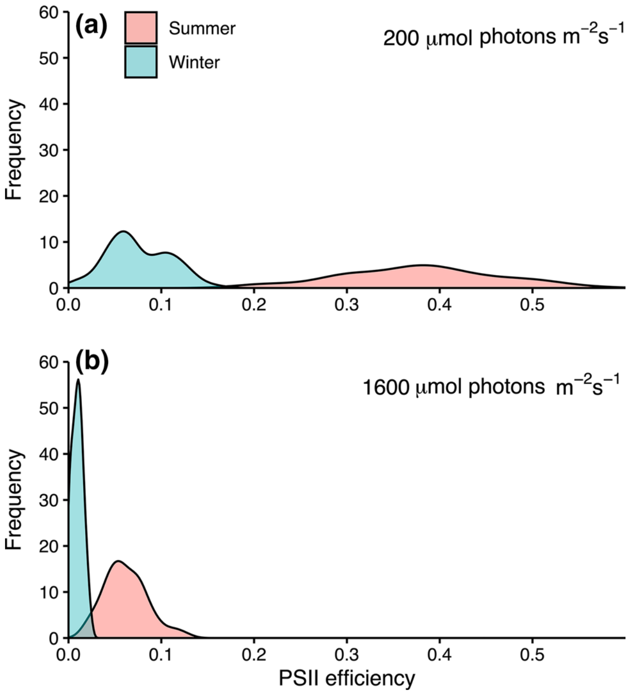


Fig. 3 The frequency distribution of photosystem II efficiencies in summer (pink) and winter (blue) at 200 µmol m^{-2} s^{-1} (**a**) and at 1600 µmol m^{-2} s^{-1} (**b**) $N = 70$

seasonal difference in Z/(Chl$a + b$) and the seasonal difference in (V + A + Z)/(Chl $a + b$) only modestly (to $r^2 = 0.33$ and 0.23, respectively).

Though we observed some decrease in chlorophyll pool size from summer to winter in most (30) of the measured (49) individuals, sampled individuals as a whole did not exhibit a significant seasonal difference in fresh mass-based needle chlorophyll content (one-way ANOVA, $p = 0.21$; Fig. 1c), nor did we observe a significant seasonal difference in the ratio of chlorophyll *a* to chlorophyll *b* (one-way ANOVA, $p = 0.41$; Fig. S2). We recorded an increase in chlorophyll pool size from summer to winter in 19 individuals, 12 of which are members of the *Pinaceae* and seven of the *Cupressaceae*. As we hypothesized, there was no significant difference between *Pinaceae* and *Cupressaceae* chlorophyll content in either summer (one-way ANOVA, $p = 0.989$) or winter ($p = 0.068$). The chlorophyll content ranged from 0.15 µmol g^{-1} (*P. wallichiana*, winter) to 2.56 µmol g^{-1} (*T. baccata*, summer), and both the mean and median decreases from summer to winter were about 7%.

Xanthophyll cycle pool size increased from summer to winter ubiquitously on a chlorophyll basis (Fig. 1d) and increased in all but three individuals on a fresh-mass basis (Fig. S2). There was no significant difference between

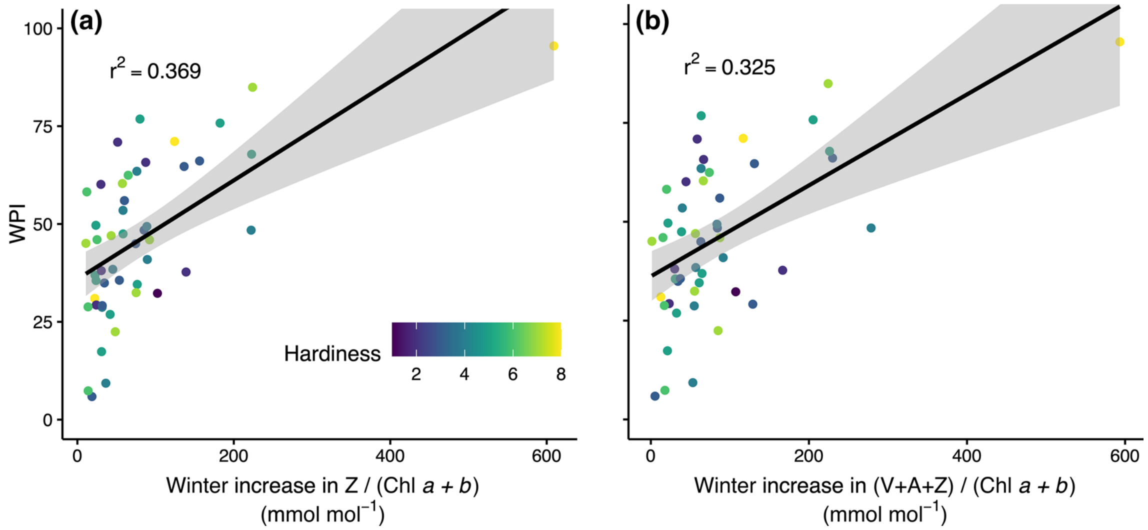


Fig. 4 Relationships between zeaxanthin content (**a**) and xanthophyll cycle pool size (**b**) on a chlorophyll basis and winter photoinhibition (WPI). USDA hardiness rating indicated by point color, as indicated. $N = 49$

Pinaceae and *Cupressaceae* winter xanthophyll cycle pool sizes; however, in summer, mean xanthophyll cycle pool size was 22% larger in *Pinaceae* than *Cupressaceae* (one-way ANOVA, $p = 0.0146$). Zeaxanthin as a proportion of the overall xanthophyll cycle pool, $Z/(V + A + Z)$, also increased from summer to winter in all individuals (Fig. S2). The majority (> 50%) of the wintertime increase in xanthophyll pool size could be attributed to an increase in Z in all but two individuals (*P. nigra 'Hornbrookiana'*, and *J. sabina 'Calgary Carpet'*). There was no significant difference in $Z/(\mathrm{Chl}a + b)$ between families in either season. When only wild-type genotypes were considered, the strength of the aforementioned correlations between pigment content and WPI increased ($Z/(\mathrm{Chl}a + b)$ versus WPI, $r^2 = 0.45$; $(V + A + Z)/(\mathrm{Chl}a + b)$ versus WPI, $r^2 = 0.40$); however, it is important to note that our study included only 12 such individuals.

Discussion

It has been known for decades that sustained energy dissipation plays a crucial role in minimizing photooxidative damage in overwintering evergreen conifers (see Adams et al. 2004). However, most studies of this protective mechanism examine one or only a few species, limiting our ability to characterize taxonomic variation in the extent of its deployment and its underlying mechanisms. Here, we examined winter acclimation of 70 mature conifer individuals growing in common-garden conditions at the Red Butte Garden, an arboretum in Salt Lake City, Utah. Arboreta offer an opportunity to compare a wide range of tree species (albeit at low replication) with little concern for the confounding effects of environmental variation, as their collections are typically diverse and relatively compact (Herben et al. 2012; Primack and Miller-Rushing 2009).

We found evidence of an extremely wide range of wintertime engagement of the slowly reversible component of sustained energy dissipation, despite common environmental conditions. To quantify the slowly reversible component of sustained energy dissipation, we adopted the 'winter photoinhibition' (WPI) parameter introduced by Miguez et al. (2015), which quantifies the percentage decrease in dark-acclimated F_v/F_m from summer to winter. WPI ranged from 6 to 95%, indicating that common environmental conditions can induce nearly complete downregulation of photochemical light use in one evergreen taxon and almost no engagement of sustained energy dissipation in another. While previous meta-analyses (Miguez et al. 2015; Esteban et al. 2015) have shown comparable breadth in WPI, our study is the first demonstration that large variation in the engagement of sustained energy dissipation can be observed between individuals grown under common conditions.

We generally focused our analyses of pigment content on pigment:pigment ratios rather than pigment content expressed on a needle fresh mass basis. This is primarily because key pigment:pigment ratios proved better predictors of WPI, but also because conifer species vary in the degree to which their needle water content varies seasonally (Little, 1970; Hatcher, 1990). This can be especially so for current-year needles, which were the focus of our study (Hatcher 1990). Differing needle water content confounds pigment content that is expressed on a fresh mass basis.

The pigment parameter most strongly correlated with WPI was the difference in $Z/(\mathrm{Chl}\ a + b)$ between summer and winter ($r^2 = 0.37$). Thus, Z alone was a better predictor of WPI than was Z + A, a more commonly used measure of xanthophyll cycle carotenoids involved in energy dissipation (Adams et al. 1995; Verhoeven et al. 1999). One interpretation of this result is that evergreens that engaged in sustained energy dissipation did so primarily via the accumulation of Z. It is notable, however, that this correlation accounts for little more than one-third of the variation in WPI we observed across individuals, which may suggest that the relationship between sustained energy dissipation and Z/(Chl$a+b$) may vary across taxa or that mechanisms not involving Z contribute to winter photoprotection (see, for example, Bag et al. 2020 and Yang et al. 2020).

Our findings suggest that remotely sensed vegetation indices, such as PRI, CCI, G_{cc}, and GRVI, which are sensitive to seasonal changes in foliar carotenoid content (Wong and Gamon 2015a and 2015b, Gamon et al. 2016, Seyednasrollah et al. 2020) may be generally useful in quantifying seasonal changes in pigment content across confer forests of different species composition. We did not find evidence of a consistent wintertime decrease in evergreen chlorophyll content, indicating that evergreen conifers, in general, do not decrease the amount of light absorbed by photosynthetic tissue during winter, when cold temperature reduces photochemical energy use. This finding has important implications in the interpretation of remotely sensed solar-induced fluorescence (SIF), which has been shown to decrease in winter in evergreen needleleaf forests (Magney et al. 2019). Decreases in the quantum yield for chlorophyll fluorescence may thus be a prevailing mechanism for seasonal decreases in SIF emitted by evergreen forests, and wintertime deployment of sustained energy dissipation likely underlies this effect (see Magney et al. 2019).

Our data allowed us to compare the seasonality of WPI and pigment content of the *Cupressaceae* and the *Pinaceae* families. Both plant families exhibited similarly broad ranges in WPI, and comparable positive correlations between WPI and winter Z content. Thus, as we hypothesized, we found no evidence for patterns in sustained energy dissipation associated with this family-level taxonomic affiliation. This may suggest that the alleviation of photooxidative stress in winter via Z accumulation evolved prior to the divergence of these two families.

Perhaps most surprisingly, the wide variation in WPI we observed did not appear to be related to temperature hardiness ratings (Fig. 2, $r^2 = 0.01$). Our study population includes individuals placed in climatic hardiness zones one (< − 45.6 °C) to eight (− 1.1 to − 6.6 °C). Red Butte Garden is located in a region with a climatic hardiness of seven, and contains no individuals growing outdoors with climatic hardiness designations of nine, ten or eleven (the most sensitive to cold), presumably because such individuals could not survive the local winter conditions. For terrestrial plants, the rate of photosynthesis falls to zero near 0 °C (Leverenz and Öquist 1987; Nippert et al. 2004; Körner 2013) Thus, at any temperature below freezing, all light absorbed by chlorophyll would be in excess, regardless of a individual's hardiness rating. This may explain why relative resistance to photo-oxidative damage via sustained energy dissipation is not a primary feature that distinguishes plants with differing climatic hardinesses. Instead, homeoviscous membrane adaptations and biochemical and ultrastructural mechanisms that enable plants to maintain membrane integrity and resist or withstand freezing of water in various celluar and extracellular compartments (Kalberer et al. 2006; Strimbeck et al. 2007; reviewed in Strimbeck et al. 2015) may distinguish plants along the hardiness continuum.

Boreal conifer-dominated forests account for around 10% of the forested area globally (Keenan et al. 2015) and evergreen conifers can be found in large extent at temperate latitudes. Our results confirm the relationship between the wintertime accumulation of zeaxanthin and wintertime decrease in photosynthetic quantum efficiency (F_v/F_m), consistent with engagement of sustained energy dissipation. Zeaxanthin content on a chlorophyll basis proved to be the most predictive, although it explained only slightly more than one-third of the remarkably broad variation in chlorophyll fluorescence manifestations of sustained energy dissipation (*i.e.,* WPI). Zeaxanthin per unit chlorophyll increases in winter due to increases in the conversion state and overall size of the xanthophyll cycle pool. Traits, such as taxonomic family affiliation and temperature hardiness, did not affect WPI. As our understanding of the mechanisms underlying wintertime photoprotection and sustained energy dissipation grows, future studies should seek to identify how these mechanisms manifest across the diversity of extant conifers.

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Author contribution statement DRB conceived of the study, which he designed with BAL. AW-M, MAG, DB, and AL performed chlorophyll fluorescence analyses. AW-M, DB, JSR and ES characterized needle chlorophyll and carotenoid composition. AW-M and CS led statistical analyses. AW-M conceived of and created figures. AW-M wrote the manuscript; all other authors provided editorial advice.

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Data availability Study data available via the Bowdoin Digital Commons.

Declarations

Conflict of interest The authors have no conflicts of interest or competing interests.

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