

Minireview

Winter leaf reddening in 'evergreen' species

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Received: 30 November 2010

Accepted: 8 January 2011

Summary

New Phytologist (2011)

doi: 10.1111/j.1469-8137.2011.03662.x

Key words: anthocyanins, carotenoids, cold, evergreen, red leaves, rhodoxanthin, winter.

Leaf reddening during autumn in senescing, deciduous tree species has received widespread attention from the public and in the scientific literature, whereas leaf reddening in evergreen species during winter remains largely ignored. Winter reddening can be observed in evergreen herbs, shrubs, vines and trees in Mediterranean, temperate, alpine, and arctic regions, and can persist for several months before dissipating with springtime warming. Yet, little is known about the functional significance of this colour change, or why it occurs in some species but not others. Here, the biochemistry, physiology and ecology associated with winter leaf reddening are reviewed, with special focus on its possible adaptive function. Photoprotection is currently the favoured hypothesis for winter reddening, but alternative explanations have scarcely been explored. Intraspecific reddening generally increases with sunlight incidence, and may also accompany photosynthetic inferiority in photosynthetically 'weak' (e.g. low-nitrogen) individuals. Red leaves tend to show symptoms of shade acclimation relative to green, consistent with a photoprotective function. However, winter-red and winter-green species often cohabitate the same high-light environments, and exhibit similar photosynthetic capacities. The factors dictating interspecific winter leaf colouration therefore remain unclear. Additional outstanding questions and future directions are also highlighted, and possible alternative functions of winter reddening discussed.

Introduction

Leaf reddening is a dramatic, natural display that can be observed in all ontogenetic stages and seasons, of which autumn has drawn the most attention (Archetti *et al.*, 2009). Reddening of new growth in young, developing leaves during spring and summer is more subtle, although it too has received a fair amount of study (Juniper, 1994; Karageorgou & Manetas, 2006; Hughes *et al.*, 2007; Karageorgou *et al.*, 2008). Winter leaf reddening (Fig. 1a–d) appears to be similarly common, especially in broadleaf,

angiosperm evergreen species at colder altitudes and latitudes, yet has received far less experimental attention (N. M. Hughes, pers. obs.). Here, the term 'winter reddening' is used to describe any change in leaf colour resulting from accumulation of red pigments before or during winter, which persists until the following spring. Winter reddening occurs irrespectively of reddening during senescence or development (e.g. some winter-red species have yellow senescing leaves, while some winter-green species have red), suggesting that its function is specific to the winter season. Leaf colour may appear brown, red, purple or orange,

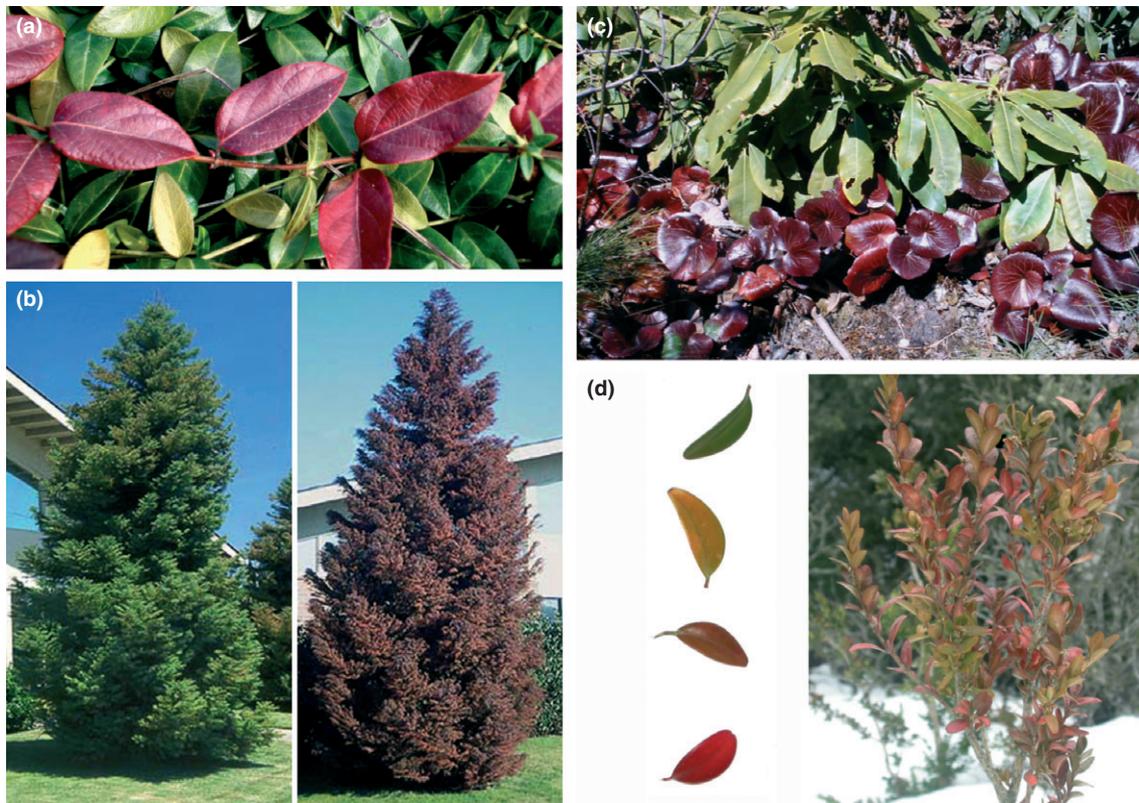


Fig. 1 Plants exhibiting winter colour change. (a) Co-occurrence of red-leaved (anthocyanic) *Lonicera japonica* and green-leaved *Vinca minor* in the same soil and light environment in the Appalachian mountains, USA; (b) summer (green) and winter (rhodoxanthin-containing) leaves of *Cryptomeria japonica* 'Elegans'; (c) co-occurrence of red-leaved (anthocyanic) *Galax urceolata* and green-leaved *Rhododendron maximum* in the Appalachian mountains, USA; (d) range of winter leaf colours of *Buxus sempervirens*, coinciding with winter accumulation of red carotenoids. Photo credits: N. M. Hughes (a, c), P. Breen, Oregon State University (b), and J. I. García-Plazaola & K. Hormaetxe (d).

depending on relative concentrations of other pigments (Hormaetxe *et al.*, 2004; Fig. 1d). In many evergreen species leaf reddening is winter-transient, and leaves become green again with springtime warming. Such leaves may persist, and undergo winter reddening again, for one or two additional winter seasons (e.g. Nikiforou & Manetas, 2010). Leaves of other winter-red species senesce while still red at winter's end, concomitant with a flush of new, green leaves. Although not technically evergreen (i.e. leaf life-span > 1 yr), these latter types of leaves will be included in the 'winter-red' category as well because they remain photosynthetically active during winter.

The sparse research on winter reddening is likely a result of a combination of: (1) the mistaken assumption that red winter leaves are undergoing senescence, similar to autumn leaves; the (2) inconspicuous appearance of many winter-red evergreens, which are commonly herbs, vines and groundcover, and escape notice against a backdrop of brown leaf litter; and (3) lack of an immediate agricultural or human application. Yet, evergreens may nevertheless represent an ideal system for studying the fundamental processes involved in leaf reddening. For example, the duration for which an individual, evergreen leaf remains red

during winter is much longer than that of a senescing or developing leaf (i.e. months vs weeks) (Sanger, 1971; N. M. Hughes, pers. obs.). Also, while senescing and developing leaves are undergoing continuous biochemical and physiological transition (and often at asynchronous times), winter-red leaves maintain relative physiological stasis throughout the winter, providing better control for repeated measurements between and within species (e.g. Hughes & Smith, 2007a; Hughes *et al.*, 2010). Lastly, because winter leaf reddening can be observed in Mediterranean (Zeliou *et al.*, 2009; Nikiforou & Manetas, 2010), temperate (Hughes & Smith, 2007a), arctic (Oberbauer & Starr, 2002), and alpine regions (Williams *et al.*, 2003; Hacker & Neuner, 2006), it can be a convenient system to utilize for local field studies.

Winter reddening: biochemistry and physiology

In angiosperms, anthocyanins are the most common pigment reported in winter reddening, while in gymnosperms, red carotenoids (most often rhodoxanthin) predominate (Weger *et al.*, 1993; Han *et al.*, 2003). Both pigments are observable in either group (e.g. Krol *et al.*, 1995;

Hormaetxe *et al.*, 2004), although they have never been observed to occur simultaneously. Betalain pigments impart reddening exclusively in Caryophyllales, and have not yet been reported in winter reddening. Beyond the very rough delineation between angiosperms and gymnosperms, no phylogenetic characterization of winter leaf colouration has been undertaken.

Anthocyanins and red carotenoids have been reported in adaxial (upper) and abaxial (lower) mesophyll during winter (Kaku *et al.*, 1992; Hormaetxe *et al.*, 2004; Hughes & Smith, 2007b). The intensity of red colouration generally increases with incident sunlight, with shade leaves often being completely green (Czeczuga, 1987; Ida *et al.*, 1991; Hughes *et al.*, 2005); the correlation between sunlight intensity and red colouration is observable in other seasons and life stages as well (Chalker-Scott, 1999; Feild *et al.*, 2001). When red and green phenotypes of a single species co-occur under apparently identical, high-light field conditions, red colouration appears to coincide with a photosynthetic handicap in the red-leaved phenotype (discussed in further detail in ‘Why are some species red and others green during winter?’, Zeliou *et al.*, 2009; Nikiforou & Manetas, 2010); however, this is not always the case (Han *et al.*, 2003). Reddening of the lower leaf surface is generally less intense than reddening of the upper surface, unless leaves are inverted by an external force (e.g. fallen branches or leaf litter; Hughes & Smith, 2007b) or natural folding movements (e.g. hydronastic closing of leaf rosettes; Hacker & Neuner, 2006), resulting in increased irradiance on the abaxial surface.

Cell structure of red leaf tissue varies depending on whether reddening is caused by anthocyanins or carotenoids (Fig. 2). Because of the vacuolar location of anthocyanin, chloroplasts of anthocyanic cells generally remain intact and functional throughout the winter, and chlorophyll content and leaf photosynthesis may match or surpass summer levels, especially on warmer winter days (Hacker & Neuner, 2006; Hughes & Smith, 2007a). By contrast, synthesis of red carotenoids corresponds with conversion of chloroplasts to chromoplasts, degradation of chlorophyll and thylakoid membranes, and accumulation of carotenoids in lipid bodies within or outside of chromoplasts (Ida *et al.*, 1991;

Hormaetxe *et al.*, 2004). During this process, the photosynthetic capacity of the plastid is lost, although cells with functional chloroplasts may persist in more abaxial tissues, allowing leaves to maintain photosynthetic activity (Fig. 2a; Han *et al.*, 2004). This process is reversible, and chromoplasts may reassemble into chloroplasts when returned to warm temperatures (Hormaetxe *et al.*, 2004).

The precise cascade of environmental, biochemical, and molecular cues that initiates winter reddening has not yet been specifically described, although much of the general research on the induction of leaf reddening can provide an indication of the mechanistic processes that might be involved. Cold temperatures (near or below freezing) have been associated with increased transcription of genes involved in anthocyanin biosynthesis (see citations in Chalker-Scott, 1999; Ruelland *et al.*, 2009) and also rhodoxanthin accumulation and formation of chromoplasts (Weger *et al.*, 1993). However, the mechanism by which plants ‘sense’ cold temperatures remains unknown (Ruelland *et al.*, 2009). Increased cytosolic Ca^{2+} is known to be one of the major upstream signaling events involved in cold sensing and response (Ruelland *et al.*, 2009), and activities of the Ca^{2+} -binding protein calmodulin (CaM) and Ca^{2+} -ATPase have been associated with anthocyanin biosynthesis (Chang-Quan *et al.*, 2005).

Cold acclimation is also often accompanied by accumulation of sugars and other solutes, which contribute to freezing point depression and osmotic adjustment. Both low water potentials and increased sugars are known to induce anthocyanin synthesis (citations in Chalker-Scott, 2002). Hughes *et al.* (2005) induced anthocyanin synthesis in the winter-red *Galax urceolata* without cold temperatures by severing major leaf veins, which resulted in accumulation of carbohydrates and anthocyanins in tissues upstream of the injury. Similarly, simply placing excised leaves in sugar solutions under moderate to high irradiance is sufficient to induce anthocyanin production in many species (Onslow, 1925). Accumulation of sugars also corresponds with conversion of chloroplasts to chromoplasts in citrus peels (Huff, 1984), but this relationship has not yet been demonstrated in leaves, to my knowledge.

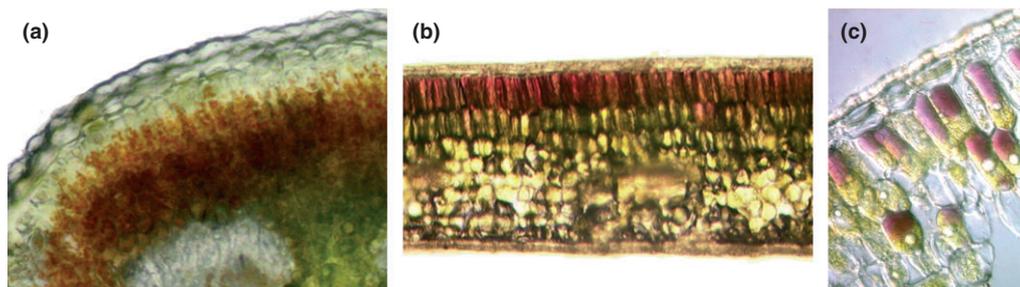


Fig. 2 Cellular compartmentalization of red pigments in evergreen leaves. (a) Red carotenoids in chromoplasts of adaxial mesophyll cells in *Buxus sempervirens*; (b) anthocyanins in vacuoles of palisade mesophyll in *Leucothoe fontanesiana*; (c) close-up of vacuolar anthocyanins in *Rhododendron* spp. Photo credits: J. I. García-Plazaola & K. Hormaetxe (a), and N. M. Hughes (b, c).

Hydrogen peroxide (H₂O₂) is also known to function as a regulatory molecule in stress perception and signal transduction, including anthocyanin synthesis (Vanderauwera *et al.*, 2005). Exposure of leaves to high light under cold temperatures causes an increase in reactive oxygen species (ROS), including H₂O₂ (Ruelland *et al.*, 2009). ROS have also been shown to induce chloroplast transformation into chromoplasts in bell pepper (*Capsicum annuum*) fruits (Bouvier *et al.*, 1998), but this has not yet been demonstrated in leaves.

The considerable overlap in induction cues that are simultaneously present during winter makes it difficult to determine which specific cues (or combination of cues) are proximately responsible for winter reddening. Perhaps more puzzling is the lack of reddening in green-leaved evergreen species, which frequently occur in the same soil and light environments as red-leaved species (Fig. 1a,c), and therefore, likely, experience many of same general physiological stresses (Fig. 3) (discussed in more detail in ‘Why are some species red and others green during winter?’).

Function of winter leaf reddening

Photoprotection

The adaptive function of winter leaf colour change is still a matter of debate. Classical physiological explanations for leaf reddening, namely, sunlight attenuation and antioxidant protection (collectively termed ‘photoprotection’), have received the most experimental attention, but may represent only one of many possible functions (Gould, 2004). During winter, the combination of cold, dry, and bright sunlight conditions can result in excess energy capture relative to processing, photoinhibition of photosynthesis, formation of ROS, and greater photo-oxidative damage (Ruelland *et al.*, 2009). Red pigments are thought to alleviate these stress factors by intercepting green sunlight (light attenuation), and/or neutralizing ROS directly as antioxidants, similar to their purported roles in juvenile and senescing leaves (Feild *et al.*, 2001; Hoch *et al.*, 2001; Neill & Gould, 2003; Han *et al.*, 2004; Hughes *et al.*, 2005, 2007). Consistent with a light-attenuating function, both inter- and intraspecific studies have shown that red-pigmented leaves generally exhibit symptoms of shade acclimation relative to green leaves under similar irradiance during winter – e.g. higher chlorophyll content, lower chlorophyll *a/b* ratios, higher maximum quantum yield of Photosystem II (*F_v/F_m*), and/or less sustained de-epoxidation of xanthophyll cycle pigments overnight (Krol *et al.*, 1995; Han *et al.*, 2003; Hughes *et al.*, 2005; Hughes & Smith, 2007a; Zeliou *et al.*, 2009; N. M. Hughes, unpublished data). When red and green individuals of a population co-occur under apparently identical, high-light field conditions, red colouration appears to coincide with a

photosynthetic handicap in the red-leaved phenotype (e.g. Zeliou *et al.*, 2009; Nikiforou & Manetas, 2010). Such a handicap may confound measurable photoprotective effects of red pigments *in situ* if protection afforded by reddening (if any) is insufficient to offset the photosynthetic inferiority (Zeliou *et al.*, 2009), and may result in the appearance that red and green individuals are equally fit, in terms of seed count, flower number, germination success, etc. (Nikiforou *et al.*, 2010). Comparisons of relative winter photo-oxidative stress in red vs green conspecific evergreens that do not differ in these and other important factors (e.g. light environment) are lacking in the literature.

In vivo, anthocyanins appear capable of neutralizing H₂O₂, which can cross the vacuolar tonoplast via membrane diffusion and aquaporins (Gould *et al.*, 2002; Kytridis & Manetas, 2006; Bienert *et al.*, 2006). Hormaetxe *et al.* (2004) briefly discuss the possibility that red carotenoids (especially retro-carotenoids) might be effective at quenching singlet oxygen in winter-red evergreens, although this possibility has yet to be investigated *in vivo* (see Hirayama *et al.*, 1994 for *in vitro* comparison of singlet oxygen neutralization capacity of carotenoids). An important issue that should be considered in the case of antioxidant protection during winter is the slow action of enzymatic systems under cold temperatures. *In vitro* enzymatic antioxidant assays of leaf extracts run at cold temperatures result in significantly reduced ROS neutralization compared with assays of the same extracts run at warm temperatures (Han *et al.*, 2004). ROS neutralization by anthocyanin and other flavonoids within the vacuole should remain relatively unaffected by cold temperatures, as they can interact directly with H₂O₂ without an enzyme involvement (Bienert *et al.*, 2006). However, diffusion of water (and thus H₂O₂) through aquaporins is known to be reduced under cold temperatures (< 20°C; Ionenko *et al.*, 2010), which could reduce the *in vivo* capacity for anthocyanins to neutralize ROS. Additional problems with an *in vivo* antioxidant function of anthocyanin are discussed in Manetas (2006), and include the spatial segregation of anthocyanins from the main site of ROS production (the chloroplast), and the availability of other, more potent antioxidants at higher concentrations relative to anthocyanin. The *in vivo* antioxidant performance of vacuolar, nonenzymatic antioxidants at cold temperatures has not been tested, to my knowledge, nor has the relative importance of ROS scavenging by flavonoids vs enzymatic antioxidants at varying temperatures. It is possible that anthocyanins may play a greater role in ROS scavenging at low temperatures, when enzymatic antioxidant systems are more impaired.

Leaf temperature

Increased absorption of green light by red pigments might also alleviate low-temperature stress by increasing leaf tem-

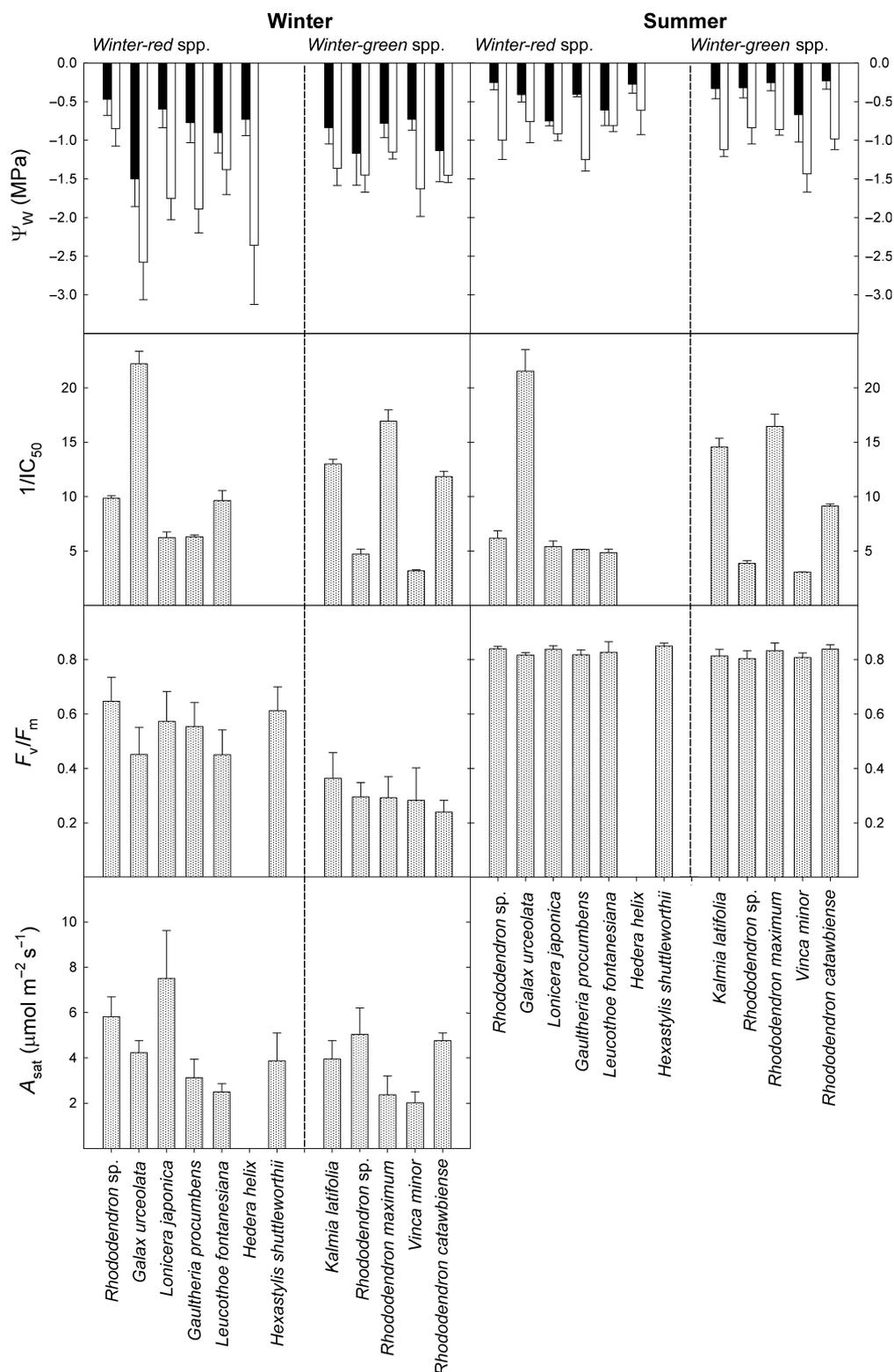


Fig. 3 Comparative seasonal physiological parameters of co-occurring winter-red (anthocyanic) and winter-green (acyanic) angiosperm evergreen species in the Appalachian mountains, USA. From top to bottom: field water potentials (Ψ_w ; closed bars, pre-dawn; open bars, midday), low-molecular-weight antioxidants ($1/IC_{50}$), pre-dawn maximum quantum yield efficiency of photosystem II (F_v/F_m), and photosynthetic capacity on a warm winter day (A_{sat}). Species to the left of the dashed lines are winter-red, and those to the right are winter-green. Note that leaves of all species are green during summer. Data depicted are from Hughes & Smith (2007a), Hughes *et al.* (2010) and N. M. Hughes (unpublished data).

perature during the day. Previous studies have been unable to demonstrate a warming effect *in situ* associated with red pigmentation (citations in Chalker-Scott, 1999; Lee *et al.*, 2003), but these studies examined leaf temperature effects under relatively mild temperature conditions (i.e. air temperatures > 8°C). Leaf energy balance models predict that increased photon absorption is likely to have the greatest effect on increasing leaf temperature (T_{Leaf}) at sub-freezing air temperatures, when transpirational cooling (i.e. stomatal conductance) and thermal conductivity of air are lowest. The effect should be most pronounced in low-wind microhabitats and in plant tissues with greater mass per unit area, such as cones and leaf buds – both of which are often red or purple, especially at high elevations. No studies have yet examined the temperature effects of red pigments in plant tissues under freezing or subfreezing conditions.

Osmotic effects

The accumulation of anthocyanins during seasons or conditions characterized by drought and freezing stress has led some to suggest that anthocyanins may be directly involved in osmotic adjustment and freezing point depression by acting as an osmolyte (Chalker-Scott, 1999, 2002). It is impossible for red carotenoids to function in this way, as they are hydrophobic molecules clustered in large plastoglobules. Most evidence for an osmotic function of anthocyanins is correlative – anthocyanin synthesis coincides with the onset of cold tolerance, and is inducible under high salinity, drought, and sugar treatments (see citations in reviews by Chalker-Scott, 1999, 2002), but the extent to which anthocyanins affect the water potential and/or freezing point of the cytosol has yet to be empirically tested. Both intra- and interspecific *in vivo* studies that address this topic (although few) have yielded results generally inconsistent with an osmotic function. Kaku *et al.* (1992) found no significant difference in the NMR relaxation time, T₁ (which is positively, linearly correlated with plant water potential; Veres *et al.*, 1991), of red and green (sun and shade) leaves of three winter-red species. Hughes *et al.* (2010) reported overlap in osmotic potential at full turgor ($\psi_{\pi,100}$) and field water potential (ψ) between red- and green-leaved evergreen species (Fig. 3), suggesting that anthocyanins are not necessary constituents of osmotic adjustment, and that alternative (and probably less costly) solutes can result in similar $\psi_{\pi,100}$.

Excess energy and/or photoassimilate sink

Another physiological hypothesis for winter reddening that has received little direct study is the hypothesis that phenylpropanoids (especially flavonoids) function as a temporary sink for reduced carbon, and act as an ‘energy escape valve’

by consuming ATP and NADPH during their biosynthesis (recently reviewed in Hernández & Van Breusegem, 2010). Cold temperatures, stomatal closure, and frozen soils inhibit energy processing, carbohydrate metabolism, growth, and export to sink tissues, which can result in reduced availability of oxidized electron acceptors and feedback inhibition of photosynthesis (Ruelland *et al.*, 2009). Anthocyanins are built on a carbon skeleton with varying numbers of sugar residues, and their biosynthesis therefore could conceivably function as a metabolically inert carbon sink until carbohydrate export and metabolism are restored. As previously mentioned, increased sugar content induces anthocyanin synthesis in many species, and anthocyanins dissipate when sink limitations are lifted (springtime warming). However, it is unknown to what degree (if any) anthocyanins relieve this potential source–sink imbalance. Hughes *et al.* (2005) examined the relationship between total nonstructural carbohydrates (TNCs) and anthocyanin content in the evergreen herb *Galax urceolata*. Contrary to a carbon-sink function, leaves of outdoor potted plants accumulated up to six-fold greater concentrations of TNCs than their clone-derived field counterparts (probably as a result of colder soils and shorter roots), but synthesized only 25% of the anthocyanin.

Coevolution with mammalian herbivores

Recent studies on autumn and juvenile leaves suggest that red pigments reduce leaf damage either by making leaves less palatable or less visible to animals lacking a red visual receptor (camouflage), or by signalling low leaf quality (Archetti, 2000; Archetti *et al.*, 2009). Most of these studies have focused on plant–insect interactions, which are generally less frequent during winter. However, many mammals (most of which also lack a functional red receptor) continue to forage during the winter, exhibit clear preference and avoidance of certain plants, and respond negatively to unpalatable secondary metabolites (Swihart & Bryant, 2001). Perhaps winter reddening has evolved as a strategy to deter herbivory by mammals during winter, subsiding during warmer months along with reduced herbivory pressure as softer, more palatable deciduous foliage becomes increasingly available. This explanation for winter leaf reddening has not been tested, and would certainly add an interesting angle to the coevolution hypothesis for red leaves.

Why are some species red and others green during winter?

Many outstanding questions remain as to why some species synthesize red pigments during winter while others (most conspicuously conifers) do not. A lack of redness does not necessarily correspond with an inability to synthesize red

pigments, as many winter-green species produce red pigments in other tissues or ontogenetic stages, for example developing leaves, flowers, stems, cones, petioles and senescing leaves (Hughes & Smith, 2007a). The lack of winter leaf reddening in species with the capacity for synthesis of red pigments suggests that they are simply not advantageous for these species during winter.

The majority of studies that have attempted to explain winter redness vs greenness have focused on stress physiology, and assume that red-leaved plants synthesize red pigments to offset some physiological handicap. For example, because the relative 'need' for photoprotection increases with limitations in energy processing, reddening might be expected to occur in individuals or species with diminished capacity for winter photosynthesis and/or energy dissipation. Indeed, many intraspecific (and some interspecific) studies have shown that winter-red leaves exhibit one or more of the following signs of photosynthetic 'weakness' relative to winter-green leaves: lower leaf nitrogen, lower photosynthetic capacity, and greater photoinhibition of photosynthesis (intraspecific studies: Hormaetxe *et al.*, 2004; Kytridis *et al.*, 2008; Zeliou *et al.*, 2009; Nikiforou & Manetas, 2010; interspecific studies: Williams *et al.*, 2003). However, others have found that winter-red leaves have significantly *higher* photosynthesis and *lower* sustained photoinhibition on average on warm winter days relative to winter-green leaves, and/or comparable ranges of total low molecular weight antioxidants (LMWA), leaf nitrogen, and xanthophyll pigments throughout the year (intraspecific studies: Han *et al.*, 2003; interspecific studies: Oberbauer & Starr, 2002; Hughes & Smith, 2007a; N. M. Hughes, unpublished data; Fig. 3). Clearly, based on the small number of field studies available and their conflicting results, we cannot yet implicate photosynthetic limitation as the cause of winter reddening.

The relative need for photoprotection also depends largely on sunlight absorption. Simple measurements of leaf and plant form could yield valuable insight into relative vulnerability to high sunlight incidence, yet no studies have surveyed winter reddening according to such factors as leaf size, leaf angle, or plant form. These factors can dramatically affect leaf and plant microclimate in terms of sunlight absorption and temperature, resulting in dramatic differences in realized winter stress, even between neighbouring plants (Smith & Hughes, 2009). Silhouette to total leaf area ratio (STAR), for example, would be interesting to measure according to winter leaf colour for various species to determine whether sunlight absorption can explain some large-scale trends in winter leaf colouration (i.e. red broadleaves vs green needles).

In addition to photosynthetic factors, low leaf water potentials (ψ) imposed by seasonal drought and freezing stress can also increase vulnerability to photo-oxidative damage by causing chlorophyll degradation, increased pho-

torespiration, and increased ROS formation, thus rendering low ψ plants (i.e. drought tolerators) in greater need of photoprotection (citations in Hughes *et al.*, 2010). Hughes *et al.* (2010) compared field ψ , osmotic adjustment, soluble sugars, and cell wall hardening of six winter-red (anthocyanic) and five winter-green temperate angiosperm species. Considerable overlaps between winter-red and winter-green species were observed for all parameters measured, suggesting that low ψ and drought tolerance alone are not sufficient to explain redness and greenness in temperate evergreens during winter.

Conclusion

While reported results are not always consistent with a photoprotective function of red pigments in evergreens, this hypothesis nevertheless remains the current front-runner as the leading adaptive explanation for winter reddening. Winter-red leaves do consistently appear to exhibit symptoms of shade adaptation relative to winter-green leaves, although whether this shade effect is the primary function, or merely a secondary effect of some other, unknown function, is unclear. Furthermore, why some species synthesize red pigments in winter leaves while others do not remains a mystery. Given that multiple physiological/environmental stress factors have been associated with leaf reddening, one might suppose that winter colour change could be attributed to any one of several possible physiological limitations associated with increased vulnerability to high light (e.g. low photosynthetic capacity, low antioxidant pools, or low leaf ψ during winter). Even this explanation fails, however, when one considers that many evergreen species exhibit one to several of these characteristics during winter, and yet do not synthesize red pigments (Fig. 3). For example, of the 12 angiosperm evergreen species I have studied in the Appalachian mountains, USA, the invasive, ornamental groundcover *Vinca minor* L. is among the species exhibiting the lowest photosynthetic capacity (A_{sat}), greatest sustained photoinhibition of photosynthesis (lowest pre-dawn F_v/F_m), lowest seasonal pools of protective LMWA, and greatest acclimation to drought stress (greater cell wall hardening ϵ , and low $\psi_{100,\pi}$) under high-light field conditions during winter – yet, it does not synthesize red pigments in its leaves (citations in Fig. 3 legend). Often co-occurring with *V. minor* within these field sites is another invasive vine, the winter-red *Lonicera japonica* Thunb. (Fig. 1a). Under virtually identical soil, temperature, and sunlight regimes as *V. minor*, *L. japonica* exhibits one of the *highest* A_{sat} and F_v/F_m during winter, and exhibits only average LMWA pools and symptoms of drought acclimation. Clearly, the factors that dictate winter leaf colouration at the interspecific level are more complex than simply light environment and photosynthetic capacity. However, given that neither of these species is native to the

region in which measurements were made, it is possible that the ultimate reason(s) for their difference in winter hues could be less related to their current environment and/or winter stress physiologies, and more a product of biogeographical or evolutionary histories. Unfortunately, the lack of information regarding these and other comparative aspects of red-leafed vs green-leafed evergreens renders alternative explanations for winter colour change purely speculative at this time. Simple studies comparing historical climate and microclimate, plant morphology, biogeography, herbivory pressure, and phylogenetic relationships of winter-red and winter-green plants could yield valuable insights to explain possible patterns underlying leaf colouration during winter, and lead to a more comprehensive understanding of this seasonal phenomenon.

Acknowledgements

Thanks to Drs Kevin Gould, William Smith, Yiannis Manetas and Howard Neufeld for research advising, mentoring, and/or peer review.

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