

The Ecophysiology of Foliar Anthocyanin

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I. Abstract

The accumulation of foliar anthocyanins can be consistently attributed to a small range of contexts. Foliar anthocyanin accumulates in young, expanding foliage, in autumnal foliage of deciduous species, in response to nutrient deficiency or ultraviolet (UV) radiation exposure, and in association with damage or defense against browsing herbivores or pathogenic fungal infection. A common thread through these causative factors is low photosynthetic capacity of foliage with accumulated anthocyanin relative to leaves at different ontogenetic stages or unaffected by the environmental factor in question.

The ecophysiological function of anthocyanin has been hypothesized as: 1) a compatible solute contributing to osmotic adjustment to drought and frost stress; 2) an antioxidant; 3) a

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UV protectant; and 4) protection from visible light. Review of the internal leaf distribution of anthocyanin, of experimental evidence using seedlings, and of studies that directly investigated light absorption by anthocyanin and its development relative to recognized processes of photoprotection support the hypothesis that anthocyanins provide protection from visible light.

II. Introduction

Anthocyanins—Greek *anthos* (flower) and *kyanos* (dark blue)—are colored pigments that give flowers their characteristic red, purple, and blue hues. Less recognized is the occurrence of anthocyanins in fruits, stems, and leaves. The first section of this review considers the eco-physiological role of anthocyanin accumulation in leaves that is associated with ontogenetic (expanding and senescing leaves), abiotic (nutrient deficiency and UV light) and biotic (herbivory and pathogen infection) effects. In the second section, the hypothesis that anthocyanin can provide a photoprotective role is developed in the context of evidence for its function as an antioxidant, its distribution in leaves, and its synthesis in seedlings. The various studies that provide direct evidence for its role in light attenuation are examined.

III. Anthocyanin Accumulation

Accumulation of anthocyanin is defined as an increase in foliar anthocyanin concentration that leads to increased absolute anthocyanin content. This can be consistently attributed to a relatively small range of contexts. Anthocyanin accumulation is often observed in expanding foliage. Such foliage is not photostable because levels of light utilization and its capacity to dissipate excess energy are low, creating a relatively high risk of photodamage. Anthocyanin is also closely associated with the colors in the autumn foliage of deciduous species. Virtually all such species exhibit visually striking anthocyanins, though also carotenoids, during this stage of leaf ontogeny. Accumulation of anthocyanin is also associated with macronutrient deficiencies, particularly with intensively managed agricultural, horticultural, or forest production where the nutrients contained in produce are removed from the system and replaced through fertilizer application. Research investigating the potential effects of atmospheric ozone depletion have indicated that exposure to increased levels of UV radiation induces the accumulation of foliar anthocyanin. Accumulation of foliar anthocyanin may be linked to browsing risk by herbivores and to defense against pathogenic fungal infection.

A. EXPANDING LEAVES

Photostability of leaves during leaf expansion, measured as resistance to degradation of the photosynthetic apparatus under natural light, is attained only after a critical amount of chlorophyll has accumulated (Drumm-Herrel & Mohr, 1985). This accumulation is associated with the development of leaf anatomy and structure and coincides with an increase in photosynthetic rate (Drumm-Herrel & Mohr, 1985). Photostability is usually attained gradually during leaf expansion (Drumm-Herrel & Mohr, 1985; Tuohy & Choinski, 1990; Choinski & Johnson, 1993; Choinski & Wise, 1999), though delayed until after full expansion in many rain-forest plants (Kursar & Coley, 1992; Nii et al., 1995; Dodd et al., 1998; Woodall et al., 1998; Karim et al., 1999). Anthocyanin has been associated with both gradual (Drumm-Herrel & Mohr, 1985) and delayed (Dodd et al., 1998; Woodall et al., 1998) chlorophyll accumulation during leaf expansion. Drumm-Herrel and Mohr (1985) showed that photostability was linked to anthocyanin production and that its accumulation in expanding leaves contributed to greater photostability in *Sinapsis alba* than in *Sesamum indicum* seedlings.

B. AUTUMN SENESCENCE

It has been suggested that anthocyanin screens senescing foliage from high levels of incident light (Hoch et al., 2001), thereby reducing the potential for photoinhibition. This creates an environment for the efficient resorption of foliar nutrients during a period of restricted photoprotective capacity. Species at risk of photoinhibition during leaf senescence, such as late successional species or those occurring in cold environments, are hypothesized to employ anthocyanin synthesis as a mechanism of photoprotection (Hoch et al., 2001). In contrast, Matile (2000: 155) considered anthocyanin accumulation “to represent a kind of extravagancy without a vital function.” To support this argument, he contends that levels of anthocyanin accumulation vary between years, between individuals within a species, and within individuals and that senescence in some species—e.g., in copper varieties of beech (*Fagus sylvatica*) and hazel (*Corylus cornuta*)—is preceded by the loss of anthocyanin (Matile, 2000).

C. NUTRITION

Nutrient, and particularly nitrogen (N), deficits detrimentally affect photosynthetic function and efficiency and decrease the levels of Calvin Cycle enzymes (Terashima & Evans, 1988; Sugiharto et al., 1990). This commonly induces or enhances the accumulation of foliar anthocyanin in leaves of many plant species (Nittler & Kenny, 1976; Hodges & Nozzolillo, 1996; Kumar & Sharma, 1999; Close et al., 2000, 2001a, 2000b). Higher concentrations of anthocyanin in sheaths and leaf blades, tissues of lower photosynthetic competence than leaves, occurred in N-deficient than N-sufficient *Zea mays* (Lawanson et al., 1975). Bongue-Bartelsman and Phillips (1995) demonstrated that N stress produces effects on expression of genes encoding enzymes associated with anthocyanin biosynthesis. Phosphorus (P) deficit, which restricts phosphate levels essential to energy metabolism (Salisbury & Ross, 1992), increased anthocyanin content in a range of monocotyledonous and dicotyledonous plants (Atkinson, 1973). Maximum levels of anthocyanin accumulation in *Z. mays* occurred after 5 days under potassium (K) deficiency (K deficit impairs photosynthetic enzyme function; Bhandal & Malik, 1988), 10 days under P deficiency, and 15 days under N deficiency (Lawanson et al., 1972).

D. ULTRAVIOLET LIGHT

Exposure to ultraviolet (UV) light promotes the production of foliar anthocyanin (Lindoo & Caldwell, 1978), and it has been hypothesized that anthocyanin provides a “UV sunscreen” (Lee & Lowry, 1980). Anthocyanin isolated from juvenile foliage of *Syzygium luehmannii* and *S. wilsonii* contributed little to the total absorbance of UV-A and UV-B radiation (Woodall & Stewart, 1998), with similar findings in *Zea mays* (Beggs & Wellman, 1985) and *Lycopersicon esculentum* (Brandt et al., 1995). The occurrence of anthocyanin in palisade and /or spongy mesophyll tissues of most species investigated to date contradicts the theory that anthocyanin has a UV-B filtering role (Gould & Quinn, 1999; Lee & Collins, 2001).

Coleus blumei var. Red Wizard, which was high in anthocyanin, had higher quantum yield and photosynthetic rate when exposed to UV-B and UV-C radiation than did *C. blumei* var. Green Rainbow, which was low in anthocyanin: Each had similar chlorophyll and carotenoid characteristics (Burger & Edwards, 1996). Krause et al. (1999) have shown, using filters that separately absorb or transmit UV radiation, that photochemical efficiency of tropical rainforest understory plants was increased when either UV-A or UV-B radiation was excluded. This was particularly evident in shade leaves exposed to full sunlight. Moreover, *Pinguicula*

vulgaris, an herbaceous plant growing in open habitats, in which foliar anthocyanin accumulation had been induced by UV-B radiation, was less susceptible to cold-induced photoinhibition (Mendez et al., 1999). These results indicate that UV radiation may contribute to photoinhibition and that anthocyanin may provide photoprotection to leaf tissues exposed to increased photoinhibition induced by UV radiation.

E. HERBIVORY AND DISEASE

Foliar anthocyanin has been implicated in plant resistance to herbivory (e.g., Coley & Kursar, 1996). However, the majority of studies that have investigated such resistance show that anthocyanin accumulation is not related directly to resistance (Satyanarayana et al., 1987; Bajaj et al., 1989) and does not inhibit feeding (Quiros et al., 1977; Isman & Duffey, 1982).

In a comparison of eggplant (*Solanum melongena*) cultivars of high and low anthocyanin content, higher levels of glycoalkaloids and phenolic compounds associated with the high anthocyanin cultivar conferred resistance to the pyralid *Leucinodes arbonalis* (Bajaj et al., 1989). Blackbird (*Agelaius phoeniceus*) preferred sunflower (*Helianthus annuus*) oilseeds low in anthocyanin, but these also had low hull mass and high oil content compared with high anthocyanin seeds (Bullard et al., 1989). Anthocyanin-containing spots produced on leaves of *Sorghum halepense* were indicative of induced resistance in response to feeding by the aphid *Sipha flava*, although there was similar coloration on water-stressed plants (Costa-Arbulu et al., 2001). Fecundity was reduced when aphids were fed the previously attacked foliage but not when fed the water-stressed foliage: Artificial diets containing increased concentrations of the purified anthocyanins did not affect survival. Costa-Arbulu et al. (2001) concluded that anthocyanin was correlated with, but not responsible for, induced resistance. The feeding preference of the fungus-growing, leaf-cutting ant (*Atta columbica*) significantly decreased with increased anthocyanin content in leaves of 20 species common to their habitat (Coley & Aide, 1989). The level of anthocyanins may provide a visual cue to herbivores of levels of other compounds. Young foliage that contains anthocyanin can have relatively high levels of total phenols (Lee & Lowry, 1980; Close et al., 2001a) and low levels of chlorophyll (Choinski & Johnson, 1993; Krause et al., 1995; Dodd et al., 1998) that are linked to low levels of N (Skillman et al., 1996).

Prominent zones of intense anthocyanin accumulation often surround restricted lesions where a plant disease has been successfully contained, whereas low anthocyanin levels often occur in susceptible combinations (Heim et al., 1983). Resistance of *Zea mays* to two fungal diseases, *Colletotrichum graminicola* (Hammerschmidt & Nicholson, 1977) and *Cochliobolus heterostrophas* (Hipskind et al., 1996), was associated with increased total phenolic content. Anthocyanin accumulation occurred considerably after growth of the pathogen had stopped and in cells that were affected, but not infected, by the pathogen (Hipskind et al., 1996). These results indicate that synthesis of phenols, rather than anthocyanin, is associated with pathogen restriction and that anthocyanin accumulation may provide photoprotection to foliar tissues affected by pathogens in which other mechanisms of photoprotection are limited.

Stone et al. (2001) reported a correlation between insect herbivory and foliar anthocyanin level in *Eucalyptus saligna* and *E. paniculata*. The basis of such an association may be similar to that of anthocyanin production induced by pathogen infection (Mach et al., 2001): Wounded foliar tissues may have restricted photosynthetic capacity. This hypothesis is supported by the observation that anthocyanin levels in insect-affected foliage increase during winter (Stone et al., 2001), when cold-induced photoinhibition may further increase the requirement for photoprotection (Close et al., 2001a, 2001b, 2002).

IV. Anthocyanin and Photoprotection

Three well-characterized mechanisms, the xanthophyll cycle, antioxidant activity, and external foliar waxes, contribute to photoprotection across plant species. The xanthophyll cycle is ubiquitous in higher plants (Müller et al., 2001). In this cycle, two carotenoids, zeaxanthin and antheraxanthin, are epoxidized to a third, violaxanthin, and in so doing light energy absorbed in excess is harmlessly dissipated (Demmig-Adams & Adams, 1992). Antioxidants (e.g., glutathione, ascorbate, tocopherols) occur in plant cells and scavenge free radicals arising from electron leakage from photosynthesis (Niyogi, 1999). Levels of free radicals increase when the capacity to utilize or dissipate excess light energy is depressed, for example, under conditions of nutrient deficit or cold-induced photoinhibition (Polle et al., 1992; Polle & Rennenberg, 1996; Logan et al., 1999). External foliar waxes reflect light, thereby providing a photoprotective function (Robinson et al., 1993; Robinson & Osmond, 1994; Barker et al., 1997).

Patterns of anthocyanin development across different families and orders of plants suggest strong convergence, and it has been hypothesized that the same physiological basis has led to this convergence (Kursar & Coley, 1992). However, the ecophysiological role of foliar anthocyanin remains contentious. Leaf color has been described as a by-product of the general metabolism of flavonoids, the color being expressed through anthocyanin as one of the by-products (Lee et al., 1987). Conversely, it has been argued that anthocyanin has four important functions, as a compatible solute contributing to osmotic adjustment to drought and frost stress (Chalker-Scott, 1999), as an antioxidant (Rice-Evans et al., 1996; Yamasaki et al., 1996; Wang et al., 1997), as a UV protectant (Lee & Lowry, 1980; Burger & Edwards, 1996; Jayakumar et al., 1999), and as protection from visible light through light attenuation (Gould et al., 1995; Krol et al., 1995; Barker et al., 1997; Dodd et al., 1998; Pietrini & Massacci, 1998; Mendez et al., 1999; Neill & Gould, 1999; Close et al., 2001a). Currently, its role in light attenuation attracts the most support.

A. OSMOTIC ADJUSTMENT

It has been argued that foliar anthocyanin confers cold and drought hardiness by contributing to osmotic adjustment in leaf tissues (Chalker-Scott, 1999). Circumstantial evidence, that cold-hardy (Kakegawa et al., 1987; Leng et al., 1993; Foot et al., 1996; Oren-Shamir & Levi-Nissim, 1997) and drought-hardy (Tuohy & Choinski, 1990; Choinski & Johnson, 1993; Ronchi et al., 1997; Sherwin & Farrant, 1998) tissues contain anthocyanin, has been presented (Chalker-Scott, 1999). However, this evidence did not exclude the possibility that cold- or drought-induced photoinhibition occurred in these studies. Thus anthocyanins may have been synthesized for photoprotection in response to photoinhibition rather than osmotic adjustment. Foliar anthocyanin does not always correlate with frost hardiness (e.g., Nozzolillo et al., 1989; Toivonen et al., 1991). Leaves of *Eucalyptus nitens* seedlings exposed to freezing temperatures but protected from high light by open-topped shade-cloth shelters had negligible anthocyanin content compared with exposed seedlings (Close et al., 2001a, 2002). Chalker-Scott (1999) concludes that anthocyanin may provide photoprotection but also prevents ice nucleation and desiccation through osmotic effects. It is not clear how this mechanism may explain anthocyanin accumulation under low but not freezing temperatures (Tan, 1979; Armitage & Carlson, 1981; Cristie et al., 1994; Boo et al., 1997).

B. ANTIOXIDANTS

Anthocyanin functions as an antioxidant *in vitro* (Rice-Evans et al., 1996; Yamasaki et al., 1996; Wang et al., 1997) and, by implication, *in vivo* (Yamasaki, 1997). Yamasaki (1997)

argued that the production of damaging reactive oxygen intermediates, by high light intensity and/or abiotic stress, provided evidence for an antioxidant role of anthocyanin in the foliage of stressed plants. However, in two field studies (Grace et al., 1998; Close et al., 2001a), seasonal variation in anthocyanin levels was not associated with variation of foliar compounds of proposed antioxidant capacity.

C. LIGHT ATTENUATION

1. *Internal Leaf Distribution*

Anthocyanin is widely distributed in the palisade and/or spongy mesophyll cells of many plant species (Gould & Quinn, 1999; Lee & Collins, 2001). It absorbs light between ca. 400 and 600 nm (Pietrini & Massacci, 1998; Close et al., 2001a). This suggests that anthocyanins can act to screen visible light. Sun et al. (1998) have shown that green light can drive carbon fixation below the palisade layer, where the spongy mesophyll can contribute as much as 40% of carbon dioxide carboxylation (Nishio et al., 1993). Chloroplasts in outer leaf layers are well adapted to excess light absorption through the activity of the xanthophyll cycle (e.g., Demmig-Adams & Adams, 1992). Sudden changes in levels of incident light—for example, sun flecks through an overstory—can induce excess light absorption that requires photoprotection via the xanthophylls cycle (Logan et al., 1997; Thiele et al., 1998). Shade-adapted chloroplasts of low chlorophyll a:b ratio may require photoprotection via anthocyanin during sun-fleck events to ensure that they fix significant amounts of carbon (Gould et al., 2000).

During leaf expansion (Lee & Lowry, 1980; Tuohy & Choinski, 1990; Choinski & Johnson, 1993; Woodall et al., 1998), during senescence (Matile et al., 1992; Feild et al., 2001), and in response to abiotic stress (Tevini et al., 1991; Krol et al., 1995; Burger & Edwards, 1996; Close et al., 2001a, 2002), leaves synthesize anthocyanin in, or immediately below, epidermal layers. The requirement for photoprotection is large if the leaves are exposed to high light conditions; this is consistent with anthocyanin providing a photoprotective role.

2. *Seedlings*

Seedlings provide a model system for investigation of the ecophysiological role of anthocyanin because young leaves have an inherently low capacity for light utilization due to low levels of chlorophyll (Krause et al., 1995; Barker et al., 1997; Dodd et al., 1998; Close et al., 2000, 2001b). Seedlings are also less adapted to abiotic stress such as frost- and cold-induced photoinhibition associated with radiative cooling (Jordan & Smith, 1995) and cold-air stratification (Jordan & Smith, 1994), drought, and waterlogging (Burdett, 1990; Close, 2001). Seedlings suffer “transplant shock” during acclimation to new field environments (Spunda et al., 1993; Mohammed & Parker, 1999; Close et al., 2000). Thus leaves of seedlings are often exposed to conditions that require maximum photoprotective effort.

Anthocyanin content in *Pinus banksiana* was twofold greater in seedlings exposed to 5°C compared with 15°C and in high light compared with low light under similar temperature conditions (Nozzollilo et al., 1989). Anthocyanin coloration was linked to nutrient deficit under low-temperature and high-light conditions in *P. sylvestris* (Toivonen et al., 1991). Anthocyanin content was not linked to photoinhibition or photoprotection in either of these studies. However, Krol et al. (1995) showed that the production of foliar anthocyanin is correlated with tolerance of photoinhibition in seedlings of *P. banksiana* and proposed that anthocyanin can reduce sensitivity to cold-induced photoinhibition. There was a twofold difference in quantum yield of oxygen evolution and in photochemical efficiency after transfer of seedlings from a

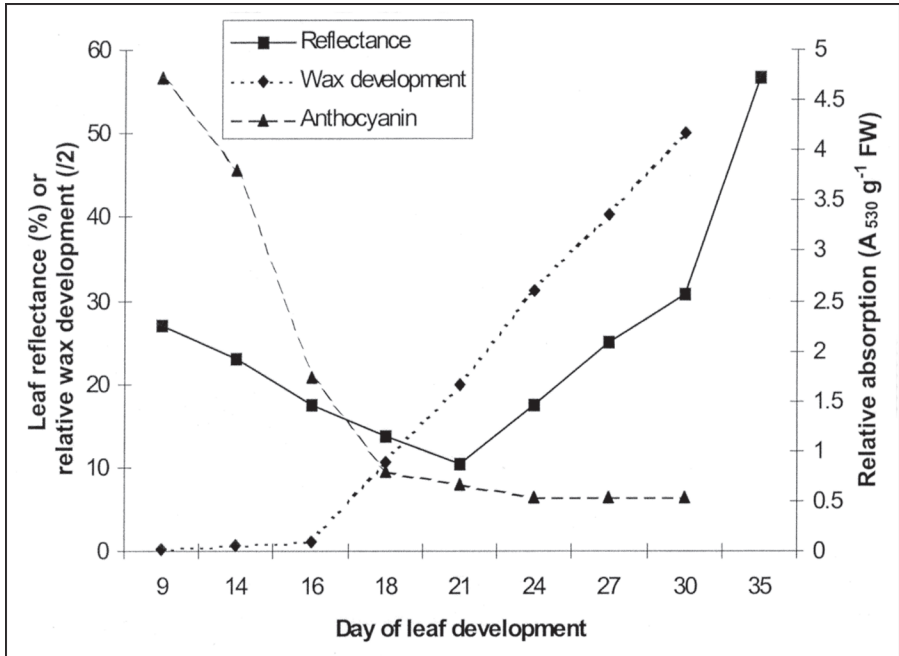


Fig. 1. Overall reflectance of incident light from 400 to 700 nm (leaf reflectance, %), an estimate of relative wax development (%/2) (wax development), and anthocyanin content (relative absorption at 530 nm per gram leaf fresh weight) of developing *Cotyledon orbiculata* leaves. Source: Adapted from Barker et al., 1997.

20°C environment to a 5°C environment (Krol et al., 1995). Photoinhibition induced by nutrient deficit was linked to the development of foliar anthocyanin in seedlings of *Eucalyptus nitens* (Close et al., 2001a). Anthocyanin synthesis and breakdown were also linked to the severity of cold-induced photoinhibition in seedlings of *E. nitens* and *E. globulus* soon after they were transplanted from the nursery to the field (Close et al., 2000). This association between anthocyanin content and the severity of photoinhibition was similarly demonstrated using shade-cloth shelters, where their subsequent removal induced photoinhibition and anthocyanin synthesis (Close et al., 2002). Such reports strongly implicate synthesis of foliar anthocyanin as a mechanism of photoprotection.

3. Does Light Absorption by Anthocyanin Provide Photoprotection?

Gould et al. (1995) have argued that anthocyanin-containing mesophyll cells protect shade-adapted chloroplasts deep within the leaf as they absorb wavelengths between 500 and 600 nm that overlap that of chlorophyll b. Subsequent studies have supported this hypothesis. Young leaves of the CAM plant *Cotyledon orbiculata* did not develop dense epidermal wax until 21–24 days of development, after which leaves reflected close to 60% of incident light (Fig. 1) (Barker et al., 1997). Prior to day 24, xanthophyll-cycle pigments per unit chlorophyll were at their highest (data not shown). Low reflectance (indicative of light attenuation) was associated with high levels of foliar anthocyanin (Fig. 1). Thus, photoprotective strategies of developing

C. orbiculata foliage include anthocyanin-mediated light attenuation and xanthophyll cycle-mediated energy dissipation during early leaf development and reflectance of light by epidermal waxes during late leaf development.

The synthesis of anthocyanin in *Zea mays* progressively increased with decreased growth temperature under constant high light and was linearly correlated with leaf absorbance across anthocyanin-absorbing wavelengths (Pietrini & Massacci, 1998). Anthocyanin synthesis in leaves of *Quintirra serrata* had enhanced absorption of light across anthocyanin-absorbing wavelengths (Neil & Gould, 1999). Gould et al. (2000) concluded that this may protect shade-adapted chloroplasts in the lower canopy from high-intensity sun flecks. In autumn-senescing leaves of *Cornus stolonifera* there was greater absorbance of light across anthocyanin-absorbing wavelengths (Feild et al., 2001).

The photosynthetic response to blue light, which is preferentially absorbed by anthocyanin, indicated that anthocyanin protects autumnal leaves from photooxidative damage, thus maximizing the efficiency of nutrient retrieval from senescing leaves (Feild et al., 2001). Foliage of *Eucalyptus nitens* with high anthocyanin levels coincided with severe foliar cold-induced photoinhibition, and leaves exhibited increased absorption across anthocyanin-absorbing wavelengths compared with that of leaves with low anthocyanin levels sampled during mild growing conditions or those protected from photoinhibition by shade cloth (Close et al., 2001a). Enhanced absorption of light by foliar anthocyanins, from a wide variety of species and ontogenetic stages, across anthocyanin-absorbing wavelengths (in association with other forms of photoprotection) strongly indicates that the ecophysiological role of foliar anthocyanins is that of photoprotection.

V. Conclusion

This analysis of anthocyanin in a range of contexts has indicated that photoprotection may be a common factor in the requirement for accumulation of anthocyanin. The internal distribution of anthocyanin in leaves supports this conclusion. Lack of anthocyanin accumulation in shaded seedlings at low temperatures suggests that anthocyanins do not contribute to osmotic adjustment. Its temporal behavior suggests that anthocyanin is not an antioxidant *in vivo*. Correlative studies of environmental effects on foliar anthocyanin strongly implicate a photoprotective role in seedlings. The contrasting anthocyanin dynamics of red/green leaves (Gould et al., 1995) and of individuals during autumn (Feild et al., 2001), the correlation of absorbance of light with anthocyanin extracts and leaves from plants exposed to different levels of cold-induced photoinhibition (Pietrini & Massacci, 1998), and anthocyanin development in conjunction with the development of other mechanisms of photoprotection such as wax (Barker et al., 1997) or xanthophyll-cycle dynamics (Close et al., 2001a, 2001b) combine to strongly indicate a photoprotective role. Thus anthocyanin possibly provides photoprotection to foliage in addition to alternative mechanisms of photoprotection or when the efficiency of these mechanisms is impaired.

Future studies at the ecological scale—for example, those investigating plant–herbivore interactions—would benefit greatly by incorporating the assessment of photoinhibition, including ontogenetic or abiotic factors that contribute to photoinhibition. Likewise, the many fertilizer response-type experiments conducted on agricultural and forestry species would benefit from an assessment of treatment effects on photoinhibition and associated changes in growth, visual symptoms, and anthocyanin accumulation. In studies of expanding or senescing leaves or of the effects of UV or excessive visible light, conclusive attribution of photoprotection to anthocyanin has thus far been complicated by parallel changes in chlorophylls and xantho-

phylls, antioxidants, and epidermal waxes. These limitations may be overcome through the use of mutants that are unable to synthesize anthocyanin. Work on genes that signal anthocyanin accumulation has largely been lacking, particularly in the context of ecophysiological investigations. Thus, analogous to studies of the xanthophyll cycle and photoprotection in mutants, it may be possible to unequivocally demonstrate that the ecophysiological role of anthocyanin is photoprotection.

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