

Nature's Swiss Army Knife: The Diverse Protective Roles of Anthocyanins in Leaves

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Received 28 June 2004; accepted 15 July 2004

Anthocyanins, the pigments responsible for spectacular displays of vermilion in the leaves of deciduous trees, have long been considered an extravagant waste of a plant's resources. Contemporary research, in contrast, has begun to show that the pigments can significantly influence the way a leaf responds to environmental stress. Anthocyanins have been implicated in tolerance to stressors as diverse as drought, UV-B, and heavy metals, as well as resistance to herbivores and pathogens. By absorbing high-energy quanta, anthocyanic cell vacuoles both protect chloroplasts from the photoinhibitory and photooxidative effects of strong light, and prevent the catabolism of photolabile defence compounds. Anthocyanins also mitigate photooxidative injury in leaves by efficiently scavenging free radicals and reactive oxygen species. Far from being a useless by-product of the flavonoid pathway, these red pigments may in some instances be critical for plant survival.

INTRODUCTION

It is no coincidence that the leaves of most higher plants are green. Chlorophyll and the associated accessory pigments allow plants to maximise use of the visible light spectrum for photosynthesis [1]. By establishing internal gradients in light capture, the green pigments enable plants to respond rapidly to changes in the spectral environment, as well as to exploit niche habitats. Equally, they provide some protection against photoinhibition and photooxidation, the damaging effects of excess quanta. Green leaves are engineered to optimise productivity.

Given the obvious benefits to their being green, why then do many plant species produce red-pigmented leaves at one or more stages in their life cycles? Red-leafed flora are common throughout all orders of the plant kingdom, from the basal liverworts to the most advanced angiosperms [2]. They occur in habitats as diverse as the Antarctic shoreline and the tropical rainforests, are as abundant in arid deserts as in freshwater lakes, and seem equally at home in the light-starved forest understorey as in the sun-drenched canopy. In many red-leafed species the manufacture of red pigments is transient, often associated with a discrete developmental stage such as in the growth flushes of tropical trees [3, 4, 5], or in the senescing autumn foliage of deciduous trees [6, 7, 8, 9]. In certain other species, however, red pigments can persist throughout the leaf's entire life span [10], or else they are induced and retained only after the plant has experienced stress [11]. Functional implications of these red pigments in plants have been the focus of a significant research output over the past decade.

For most vascular plants, red colouration in leaves is achieved by anthocyanins, predominantly cyanidin-3-O-glucoside, as a solution located in the vacuole of the plant cell. Other pigments—notably the betalains, certain carotenoids, thiarubrine A, some terpenoids, and the 3-deoxyanthocyanins—also impart reddish colours in various species; these pigments have been less well studied than the anthocyanins, but at least some of them have comparable functions in leaves [12, 13, 14, 15, 16].

The synthesis and vacuolar sequestration of anthocyanin molecules represent a considerable metabolic investment for plant cells. First, there are metabolic costs associated with enzyme production and activity; at least seven enzymes are involved in the biosynthesis of cyanidin from its precursors, 4-coumaroyl-CoA and malonyl-CoA [17]. Then there is a cost associated with the conjugation of each cyanidin molecule to a monosaccharide molecule. Finally, there are costs associated with the transport of cyanidin-3-O-glucoside into the cell vacuole via a tonoplast Mg-ATP-requiring glutathione carrier [18]. This investment suggests that the accumulation of anthocyanins in leaf cells is unlikely to be an “extravagancy without a vital function” [19]. Neither is it likely that these red pigments are simply the default product of a saturated flavonoid biosynthetic pathway, since the timing of anthocyanin production is usually tightly controlled and often occurs in tissues remote from those associated with other flavonoids [10]. On the contrary, a wealth of recent evidence, both empirical and theoretical, ascribes a remarkable diversity of functions to anthocyanins in leaves, many of them associated with stress responses and some potentially critical to a plant's survival. Anthocyanins are

arguably the most versatile of all pigments, their multifarious roles in plant stress responses stemming as much from the physicochemical property of light absorption as from their unique combination of biochemical reactivities. Recent advances in our understanding of these various functions are the subject of this review.

CONSEQUENCES OF BEING RED

Anthocyanins in vivo absorb the green and yellow wavebands of light, commonly between 500 and 600 nm [20, 21, 22, 23, 24, 25]. Foliage appears red because of the subtraction of yellow-green light from the spectrum of light reflected from the leaf's surface. Interestingly, the amount of red light that is reflected from red leaves often only poorly correlates to anthocyanin content [20]; leaf morphology and the amount and distribution of chlorophyll are apparently the stronger determinants of red reflectance. The property of anthocyanins to absorb light provides a mechanism for several important functions in leaves.

Herbivory

The red colours of anthocyanic leaves have been proposed both to attract and to repel various animal species. Burns and Dalen [26] postulated that red-orange autumn foliage of Canadian shrub species would accentuate the conspicuousness of black-coloured fruits to birds. Experimental manipulations of fruit and background foliage colours confirmed that the black-red contrast was indeed an effective enhancer of fruit-removal rates by avian dispersers. Certain insects, on the other hand, seem to preferentially avoid eating red-pigmented leaves. California maple aphids, for example, readily colonise yellow-orange leaves of Japanese maples, yet they largely ignore red-leaved individuals [27]. Similarly, leaf-cutting ants from the tropical forests of Panama browse significantly less on red leaves than on green leaves [28]. To these and other insects the anthocyanins may serve as aposematic signals of defensive commitment against herbivory [29]. Alternatively, the red pigments may simply render the leaves unpalatable. Leaves that are rich in chlorophyll as well as anthocyanin tend to be brown or even black, mimicking dead foliage or else serving to camouflage leaves against the exposed soil and litter of forest floors [30, 31, 32, 33]. Even brilliant red or scarlet leaves can appear dark to nonmammalian folivores, which lack red light receptors [5, 34]. The gains to be had from herbivore deterrence would offset metabolic costs to the plant associated with anthocyanin biosynthesis.

Protection of photolabile defence compounds

By intercepting the high-energy quanta, anthocyanic cell vacuoles can prevent important photolabile molecules from degradation by green light. An elegant example of this was described recently for the silver beachweed (*Am-*

brosia chamissonis), a composite that grows at exposed, sunny locations along the California coast. The plant holds large amounts of thiarubrine A, a potent defence compound that is toxic to insects, bacteria, and fungi [35]. Thiarubrine A is photolabile; even short exposures to visible light and/or ultraviolet radiation render it inactive [36, 37]. However, the tissues in *A. chamissonis* that contain thiarubrine A are shielded from light by a sheath of cells containing a mix of two anthocyanins, cyanidin-3-O-glucoside and cyanidin-3-O-(6'-O-malonylglucoside). The anthocyanins absorb quanta that would otherwise lead to the destruction of thiarubrine A, and thereby contribute significantly to the defensive armoury of the plant.

Protection of photosynthetic apparatus

When leaves receive more light energy than can be used in photochemistry, they show a characteristic decline in the quantum efficiency of photosynthesis, termed photoinhibition [38]. Under severe conditions the chloroplasts generate reactive oxygen species, which have the potential to destroy thylakoid membranes, damage DNA, and denature proteins associated with photosynthetic electron transport. Anthocyanins have been shown in many plant species to reduce both the frequency and severity of photoinhibition, as well as to expedite photosynthetic recovery [23, 39, 40, 41, 42, 43, 44]. In red-osier dogwood (*Cornus stolonifera*), for example, a 30-minute exposure to strong white light reduced the quantum efficiency of photosynthesis by 60% in red leaves, but by almost 100% in acyanic leaves [23]. When the plants were returned to darkness, the red leaves recovered to their maximum potential after only 80 minutes, yet their acyanic counterparts had not achieved the pretreatment state even after six hours.

Anthocyanins protect leaves from the stress of photoinhibitory light fluxes by absorbing the excess photons that would otherwise be intercepted by chlorophyll *b*. Although red leaves absorb more green light in total, their photosynthetic tissues actually receive fewer quanta than do those of acyanic leaves because the energy absorbed by the cell vacuole cannot be transferred to the chloroplasts [45]. As a result, under light-limiting environments the photosynthetic efficiencies of red leaves are often slightly lower than those for acyanic leaves [4, 22, 45, 46, 47, 48, 49]. Under strong light, however, the anthocyanins serve as a useful optical filter, diverting excess high-energy quanta away from an already saturated photosynthetic electron transport chain. Chloroplasts irradiated with light that has first passed through a red filter have been shown to generate fewer superoxide radicals, thereby reducing the propensity for structural damage to the photosystems [25]. The anthocyanins are therefore clearly a useful supplement to other nonphotochemical quenching mechanisms such as the xanthophyll cycle pigments. Recent studies involving mutants of *Arabidopsis thaliana* indicate that whereas xanthophylls have a greater role in the protection of plants from short-term

light stress, the anthocyanins can be the more effective photoprotectants over the long term [50].

The photoprotection hypothesis potentially explains why the leaves of many deciduous trees turn red in the autumn. As leaves senesce, nitrogen associated with their chloroplasts is resorbed into the branches. Anthocyanins would protect the degrading chlorophyll from damaging light levels, thereby restricting the formation of reactive oxygen that could jeopardize the resorptive process [2, 8, 9, 23, 51, 52]. Consistent with this hypothesis, nitrogen resorption has recently been shown to be more efficient in wild-type than in anthocyanin-deficient mutants of three woody species [53].

Protection from ultraviolet radiation

Interest in the flavonoid family has increased in recent years following the observation that these compounds act as sunscreens against potentially damaging UV-B radiation. Foliar anthocyanins have generally been included with other flavonoids in this UV-B protective role. Consistent with this hypothesis, the anthocyanins, particularly when acylated, absorb strongly in the UV region [54, 55], are induced or upregulated in plant tissues in response to UV irradiation [56, 57, 58, 59, 60], and mitigate DNA damage in UV-B-irradiated cell cultures [61, 62, 63]. Furthermore, certain anthocyanin-deficient mutants of *Arabidopsis* are hypersensitive to UV-B [64], and red-leafed *Coleus* varieties retain higher photosynthetic efficiencies after UV irradiation than do green-leafed varieties [49].

Notwithstanding this body of evidence, there is now a growing conviction that foliar anthocyanins cannot be primarily concerned with UV protection. Unlike the colourless flavonoids, the anthocyanins are usually located in the internal mesophyll tissue rather than in the epidermis, the optimal site for UV interception [33, 65]. Moreover, UV vulnerability often correlates only poorly to anthocyanin content. For example, an *Arabidopsis* mutant with enhanced sensitivity to UV radiation was found deficient in certain flavonoids, yet it held normal amounts of anthocyanin [66]. Similarly, the responses of *Brassica rapa* mutants to supplementary UV-B treatment were for the most part independent of anthocyanin levels in the leaves [67]. Indeed, red-leafed plants of petunia (*Impatiens*) and rice have all been observed to perform significantly worse than their green-leafed counterparts under UV-enriched environments [68, 69, 70]. Hada et al. [71] noted that DNA damage after prolonged UV treatment was substantially greater in purple-leafed rice than in a near-isogenic green line. To repair UV-damaged DNA, plants employ photolyase, an enzyme that uses blue/UV-A light to remonomerise the pyrimidine dimers. The anthocyanins in purple rice prevented the photoactivation of photolyase by absorbing some of the blue/UV-A light incident on the leaves. Thus, any short-term gain from the absorption of UV-B by anthocyanins would be offset by their property to absorb visible light and thereby limit the rate of DNA repair.

FREE RADICAL SCAVENGING

Anthocyanins diminish the oxidative load in a leaf simply by filtering out yellow-green light, since the majority of reactive oxygen in plant cells is derived from the excitation of chlorophyll. Anthocyanins are, in addition, excellent scavengers of free radicals. Purified solutions scavenge almost all species of reactive oxygen and nitrogen with an efficiency up to four times greater than those of ascorbate and α -tocopherol [72, 73, 74]. Recent experimental evidence indicates that this antioxidant potential is indeed utilised by plant cells. In *Arabidopsis*, for example, strong light and low temperatures caused more lipid peroxidation in anthocyanin-deficient mutants than in wild-type plants [50]. Similarly, upon gamma irradiation, only those *Arabidopsis* plants that contained both anthocyanin and ascorbic acid were able to grow and flower normally [75].

Microscopic examinations of wounded leaf peels have shown that red-pigmented cells eliminate H_2O_2 significantly faster than do green cells [76]. It is not clear, however, whether scavenging occurs predominantly by the red tautomers of anthocyanin found inside the cell vacuole, or else by the colourless tautomers in the cytosol. Both forms have impressive antioxidant potentials [77, 78, 79]. In a model in vitro system, the colourless tautomers of cyanidin 3-(6-malonyl)glucoside were found capable of scavenging up to 17% of the superoxide radicals generated by irradiated chloroplasts [25]. Given their proximity to the organelle sources of reactive oxygen, it may be that the cytosolic anthocyanins, rather than those in the cell vacuole, provide the greater contribution to antioxidant defence.

The degree to which anthocyanins contribute to the arsenal of low-molecular-weight antioxidants (LMWA) varies among species. In the young, red leaves of *Elatostema rugosum*, an understory herb from New Zealand, anthocyanins are the predominant phenolic component of the LMWA pool [78]. In contrast, the red- and green-leafed morphs of the canopy tree *Quintinia serrata* both hold hydroxycinnamic acids as their most concentrated LMWA [79]. Similar differences have been reported across ecotypes of wild-type *Arabidopsis* [75]. Thus it would seem that anthocyanin biosynthesis can enhance but is not usually a prerequisite for protection from oxidative stress.

AMELIORATION OF STRESS RESPONSES

The induction of foliar anthocyanins has been implicated in the acquisition of tolerance to many different kinds of environmental stressors [11, 80, 81]. Anthocyanins, for example, are associated with enhanced resistance to the effects of chilling and freezing [82, 83, 84, 85, 86], to heavy metal contamination [87, 88, 89, 90], to desiccation [91, 92, 93], and to wounding [76, 94, 95]. It is not clear at this stage whether the apparent ameliorative properties stem from one or more types of mechanism. Chalker-Scott [11, 80] provided a compelling case for

a generalised role of anthocyanins as osmoregulators in plant cells, since most types of suboptimal environments induce water stress, either directly or indirectly. Others have argued that the photoprotective [96] or the antioxidant [97] properties of anthocyanins are paramount. Regardless of their mechanism, it is clear that anthocyanins offer multifaceted, versatile, and effective protection to plants under stress. They are the Swiss army knife of the plant kingdom.

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