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## Invited Review

# Environmental Significance of Anthocyanins in Plant Stress Responses

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## ABSTRACT

Anthocyanins are water-soluble pigments found in all plant tissues throughout the plant kingdom. Our understanding of anthocyanin biosynthesis and its molecular control has greatly improved in the last decade. The adaptive advantages of anthocyanins, especially in non-reproductive tissues, is much less clear. Anthocyanins often appear transiently at specific developmental stages and may be induced by a number of environmental factors including visible and UVB radiation, cold temperatures and water stress. The subsequent production and localization of anthocyanins in root, stem and especially leaf tissues may allow the plant to develop resistance to a number of environmental stresses. This article reviews the environmental induction of anthocyanins and their proposed importance in ameliorating environmental stresses induced by visible and UVB radiation, drought and cold temperatures.

## INTRODUCTION

The characteristic red, blue and purple coloration seen in various tissues of a diverse assortment of plants is due to anthocyanins either in part (flowers and fruits) or exclusively (leaves). Anthocyanins are water-soluble pigments derived from flavonoids *via* the shikimic acid pathway. Anthocyanins may be developmentally transient, appearing only in juvenile or senescing tissues, or they may be permanent. Likewise, they may be environmentally transient, appearing and disappearing with changes in photoperiod, temperature or other signals. The photoinduction, signal transduction, gene expression and biosynthetic pathway of anthocyanins have been extensively studied and recently reviewed (1-3). The literature on their environmental induction and subsequent impact on plant survival, especially in regard to non-reproductive tissues, has not been reviewed, however. In terms of adaptive advantage, leaf anthocyanins may be the least understood group of nonphotosynthetic pigments in plants.

## MORPHOLOGICAL AND DEVELOPMENTAL ASSOCIATIONS

### Tissue location

Apart from their widely reported distribution in floral and fruit tissues, anthocyanins are also located in roots, shoots and leaves. Anthocyanins have been reported in the root cap of *Impatiens* seedlings (4) and in roots under osmotic (5) or toxin (6) stress. Stems may accumulate anthocyanins (7,8), often as a function of juvenility (9-11), osmotic stress (12,13) or cold temperatures (14-17).

Leaves of both gymnosperms (9,18) and angiosperms (17,19-34) accumulate anthocyanins in their tissues. In most cases, anthocyanins are found in or just below the upper epidermis of leaves (18,20,31,33-35). Shade plants accumulate anthocyanins in their lower epidermis, perhaps to assist in light capture (26,27). Notably, anthocyanins have been reported to occur only in the mesophyll layers of other plant species including *Mahonia*, *Viburnum* and *Rhododendron* (24) and the tropical woody genus *Syzygium* (33).

### Juvenile reddening

One of the most striking phenomena associated with anthocyanins is the juvenile reddening exhibited by a variety of plant species. Many herbaceous seedlings accumulate anthocyanins transiently, often as a result of photoinduction and always within hours or days after germination (5,36-53). Seedlings of woody species seem to require additional environmental cues such as cold temperatures (9,14,18) to accumulate anthocyanins, while their mature counterparts do not demonstrate this ability. Temperate woody perennials with juvenile reddening may show leaf flush in response to cool temperatures (54), while deciduous tropical species produce red leaves in response to drought (21,55). Yet other tropical species produce juvenile anthocyanins in response to as yet unknown signals: light-harvesting, photoprotection and antifungal defenses have been variously proposed (26,27,33,34,56).

## INDUCTION OF ANTHOCYANIN BIOSYNTHESIS

### Photoinduction

Most research on anthocyanins has focused on their photoinduction by wavelengths in the UV, visible and far-red regions. The importance of a light stimulus is further support-

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ed by the dark inhibition of anthocyanin synthesis (9,15,57), although too much radiation in the UVB region inhibits anthocyanin synthesis, probably *via* DNA damage (29,38,44).

Although photoinduction of anthocyanins has been demonstrated numerous times in both the laboratory and field, the actual photoreceptors responsible have not been clearly identified. In early research, Lindoo and Caldwell (28) theorized that far-red and UVB radiation (and therefore their photoreceptors) acted independently on anthocyanin formation in *Rumex patientia*. Further research seemed only to increase the murkiness of the debate. In reviewing the widespread phenomena of juvenile anthocyanin production in seedlings, Mohr *et al.* (48) skirt the issue by stating that "the actual induction wavelength varies among species."

Subsequent studies of various plant species and tissues have divided researchers into two camps: those who assert that anthocyanin synthesis is induced by the UVB photoreceptor (25,37,50,52,53,57-64) and those who argue that induction is through some combination of the UVB photoreceptor, phytochrome and cryptochrome (16,36,40,41,45,46,65-68). Convincing evidence for the first hypothesis is illustrated by Brandt *et al.* (37), who found a *Lycopersicon* mutant deficient in phytochrome accumulated anthocyanins *via* UVB exposure. In UVB exclusion experiments, Krizek *et al.* (25) found significant decreases in both flavonoid and anthocyanin concentrations of red-leafed lettuce. Ambasht and Agrawal (58) found field-grown *Zea mays* accumulated anthocyanins following UVB exposure. Conversely, Beggs and Wellmann (36) found some varieties of *Z. mays* to synthesize anthocyanins in response to far red, UVB or to no light at all.

In their review, Mol *et al.* (3) suggest that phytochrome is involved in anthocyanin synthesis, but that another photoreceptor is also involved. They further suggest the existence of two separate anthocyanin pathways under differential regulation. Perhaps the most reasonable hypothesis is that UVB is the only photoinducer of anthocyanins, while their relative amounts are modulated by phytochrome (50,68). Certainly the majority of the published data could be explained by this mechanism. Furthermore, anthocyanin induction might be modified by developmental stage or by environmental cues such as temperature (16).

### Cold temperature induction

Induction of anthocyanins by cold temperatures has received less attention than photoinduction, even though the evidence for this process is seen in deciduous plants every fall. Low temperature has been shown to induce anthocyanin synthesis in seedlings of *Arabidopsis* (69,70), *Sorghum* (16), *Poncirus* (17) and *Z. mays* (39), leaves of *Cotinus* (54,71) and *Pinus* (18), 1 year-old twigs of *Diospyros* (14) and ray parenchyma cells of *Fagus sylvatica* (72). Christie *et al.* (39) consider the anthocyanin biosynthetic pathway to involve *cor* (cold-regulation) genes but observe that very cold temperatures destroy the biosynthetic capability. McKown *et al.* (73) agree that there is some commonality between anthocyanin biosynthesis and freezing tolerance, in either the synthetic or regulatory pathways leading to both. It should be noted, however, that low temperatures in the absence of either visible light (23) or UVB (71) prevent anthocyanin biosynthe-

sis. As Mol *et al.* (3) conclude, the mechanism of cold induction of anthocyanins and role of light is not fully understood and they again suggest separate, or perhaps overlapping, pathways.

### Osmotic induction

A third body of information exists relative to the osmotic induction of anthocyanins. Studies with cell cultures of various species find anthocyanin accumulation resulting from osmotic stress induced by glucose (74,75), sucrose (76-81) and mannitol (74,75,78,80,82). Dilution stress was also shown to induce anthocyanins in normally pigment-free *Petunia* cell cultures (83).

Experiments in whole-plant systems have shown similar results. Anthocyanin accumulation induced by saline conditions was seen in *Z. mays* roots (5), *Morus alba* leaves (84) and the lower stems of *Casuarina equisetifolia* seedlings (12). *Arabidopsis* (85), *Terminalia catappa* (86) and *Hedera helix* leaves (11) all accumulated anthocyanins when grown in the presence of various sugars.

Obviously increased osmoticum will induce a dehydration strain on plant cells. In related studies, drought stress significantly increased anthocyanin levels in cowpea seedlings (87), resurrection plants (88) and in *Cotinus* leaves (Oren-Shamir, unpublished data). Flooded *Malus* and *Pyrus* trees showed anthocyanin increases in their leaves (89), probably in response to the secondary drought stress imposed upon leaves by depressed root function.

### Other induction factors

An assortment of other intrinsic and environmental factors have been linked to anthocyanin induction, accumulation or inhibition (3,46,69). Deficiencies in nitrogen (19,79,80), phosphorus (80,90,91) or both (92) cause anthocyanins to increase, as does exposure to lowered pH (82), methyl jasmonate (93), wounding (94), pathogen infection (95) and fungal elicitors (95). In contrast, Gläßgen *et al.* (61), Gleitz *et al.* (62) and Lo and Nicholson (96) noted suppression of anthocyanins in the presence of fungal elicitors. Differential responses to herbicides were also seen in treated seedlings, with alachlor and rimsulfuron stimulating production and trifluralin and fluometuron inhibiting anthocyanin accumulation (97).

The impact of exogenous growth regulators on anthocyanin accumulation is also unclear. Both auxins and/or cytokinins have been shown to induce anthocyanins in cell cultures (80,81) or whole plant systems (98), and others have linked gibberellins to anthocyanin production (3). However, Ronchi *et al.* (51) concluded that anthocyanin accumulation in *Z. mays* was instead linked to gibberellin inhibition. Likewise, gibberellins were seen to have no effect on anthocyanins in *Photinia* leaves (Oren-Shamir and Nissim-Levi, unpublished data). Although others have noted an inhibitory effect of abscisic acid (ABA)<sup>†</sup> (3,4), Fambrini *et al.* (42) suggest that lack of ABA prevents anthocyanin manufacture.

<sup>†</sup>Abbreviations: ABA, abscisic acid; PAL, phenylalanine ammonia-lyase.

## ADAPTIVE SIGNIFICANCE OF ANTHOCYANINS

What adaptive advantages do leaf tissues containing high levels of anthocyanin have over those tissues with lower levels? To answer this question, the relative costs and benefits of anthocyanin accumulation must be compared.

### Metabolic costs

Anthocyanin synthesis is metabolically expensive, requiring additional modifications of flavonol precursors. Their eventual degradation, such as that seen during maturation of red juvenile leaves of *Brachystegia* spp. (21), must also use energy. Another potential cost of anthocyanin accumulation is their interference with the light reactions of photosynthesis. Because of their ability to absorb blue light and reflect red wavelengths, anthocyanins in the upper epidermis or mesophyll of leaves could theoretically compete with light harvesting by chlorophyll and carotenoids. Reductions in photosynthetic rates have been noted in red-leafed varieties of *Coleus* (20) and pepper (99), spring flushing leaves of *Brachystegia* spp. (21,55) and the red juvenile leaves of several rainforest tree species (33).

### Environmental strain reduction—Photoprotection

Because they are photoinduced, researchers surmise that anthocyanins must have a photoprotective function, either against light-induced photooxidation or against UVB damage. While clear evidence exists to support the former theory, the role of anthocyanins in UVB protection is much less apparent.

In 1984 Drumm-Herrel (40) suggested that anthocyanins protect against damaging levels of light, especially high-energy blue wavelengths that can damage protochlorophyll in developing leaves. Subsequent studies have supported this theory, although proof is far from definitive. Anthocyanin accumulation during dehydration of resurrection plants (*Craterostigma wimsii* and *Xerophyta viscosa*) is thought to shield these dehydrated plants from photoinhibition (88). Similar shielding is suggested for 1 month-old *Pinus banksiana* seedlings (18), juvenile leaves of several rainforest species (33) and evergreen leaves of *Mahonia*, *Viburnum* and *Rhododendron* (24).

Whether the same protection is afforded to UVB radiation is questionable. Numerous studies (100) have implicated UVB radiation in having a significant negative impact on plant productivity, particularly in crop plants that have been bred for soft characteristics. In contrast, resistant or tolerant plants adapt to UVB stress either through photorepair of damage or radiation attenuation. This latter strategy includes thickened cuticles (101) and cell walls (102), incorporation of cell wall phenolics (102) including lignin (103,104) and increased pubescence (105). As Beggs and Wellmann (36) observed, however, anthocyanins do not absorb well in the UVB range nor could they find hard evidence for a role in UVB protection. Others have concurred with this assessment (37,106), adding that anthocyanins often occur in very low concentrations compared to other UVB-absorbing compounds and require a long exposure to UVB to be synthesized (37).

Nonetheless, there is evidence that anthocyanins do prevent UVB damage in some instances. Cell cultures of *Centaurea cyanus* were apparently protected from UVB-induced DNA damage by anthocyanins (107). Hada *et al.* (44) associated decreased anthocyanins with increased DNA damage to *Sorghum bicolor* seedlings irradiated with UVB. Both *Arabidopsis* (108) and *Zea* (109) flavonoid-deficient mutants are hypersensitive to UVB. Gitz *et al.* (43) noted that red cabbage seedlings treated with the phenylalanine ammonia-lyase (PAL) inhibitor 2-amino-indan-2-phosphonic acid were more damaged by UVB radiation than untreated controls. The PAL inhibition, of course, would also reduce other UVB-absorbing phenolics and flavonoids as well as anthocyanins. On a more speculative note, many researchers have noted the presence of anthocyanins in the upper epidermal layer of leaves belonging to a number of different plant species and associate them with UVB protection (20,21,33,34,55,56,110).

In reviewing this evidence, it is important to understand the differential absorption of covalently modified anthocyanins. Tevini *et al.* (31) noted that anthocyanins esterified with cinnamic acids do absorb UVB radiation. Likewise, Woodall and Stewart (34) mention that anthocyanins acylated with phenolics such as cinnamic acid are able to absorb higher end UVB (310–320 nm), and the absence of these compounds was directly correlated with UVB damage in red cabbage seedlings (43). Some stresses have been shown to increase the methylation (78) or acylation (90) of anthocyanins. Obviously such alterations would impact the absorption spectrum of these compounds. Similarly, absorptive artifacts could occur during improper isolation of anthocyanins, dissociating covalent bonds.

Finally, the importance of flavonoids should not be overlooked in discussions of anthocyanin production and UVB protection. Flavonoids are induced by UVB (29,31,37,61,111) and recognized as strong UVB absorbers (35,40,106). In fact, their absorption of UVB is much stronger than that of anthocyanins. Therein lies a conundrum: if one of the effects of UVB irradiation on plants is to induce UVB-protective pigments, why are anthocyanins produced instead of their colorless flavonoid precursors? It should be emphasized that anthocyanin production represents a conversion of flavonol precursors (37), which themselves are strong UVB absorbers. Perhaps the improved solubility of anthocyanins, which in contrast to other flavonoids are nearly always glycosylated (112), allow them to be stored in the vacuole more efficiently than nonglycosylated flavonoids.

### Environmental strain reduction—Cold hardiness

Freezing temperatures can inflict mechanical injury on plant cells *via* ice crystal formation or induce dehydration as liquid water becomes extracellular ice. In nature, cold-exposed tissues take several weeks to winter harden through various mechanisms including the deposition of phenolic-rich compounds such as lignin in their cell walls. These structural changes allow cells to withstand physical damage from ice formation in extracellular spaces or on epidermal surfaces. Unlike mature tissues, expanding leaves cannot cold harden by lignifying their cell walls. Many plants avoid freeze damage to sensitive tissues through water supercooling as low

as  $-41^{\circ}\text{C}$  before freezing (113). Such supercooling is often induced by increasing solute levels and has been associated with xylem ray parenchyma cells, dormant flower buds (114) and leaf tissues (115). Anthocyanin accumulation by epidermal cells in these latter tissues would decrease the osmotic potential of the cell and delay freezing *via* surface nucleators, thus protecting the leaves from late spring frosts.

Decades ago, Parker (30) linked anthocyanin appearance and disappearance to cold hardiness in *Hedera helix* leaves. Further work (116) refuted these results, demonstrating no correlation between hardiness and anthocyanin levels in *H. helix*. It should be noted, however, that these latter experiments were conducted in a greenhouse and therefore the results might not be indicative of field conditions (*i.e.* natural UVB levels). Parker (30), on the other hand, used field-grown leaf tissues.

More recently, Singh *et al.* (7) studied anthocyanin content and its relevance to cold hardiness of field-grown chickpea (*Cicer arietinum*). They concluded there was no association between stem anthocyanins and cold hardiness but did not report these data in the article. Furthermore, because this was not a controlled experiment (*e.g.* outdoor temperature fluctuations), they would not be able to assess small, but significant, differences in cold hardiness among genetic lines.

The induction of anthocyanins by chilling temperatures does suggest a protective function, and some studies are supportive of this idea. McKown *et al.* (73) suggest some commonality between anthocyanin biosynthesis and freezing tolerance, as four *Arabidopsis* mutants deficient in freezing tolerance were unable to accumulate anthocyanins. Autumn induction of anthocyanins is widely known and occurs in tandem with the onset of dormancy and cold hardiness in many woody plants. Winter-hardy tissues containing high levels of anthocyanins (14,15,71,117) generally decrease or lose these pigments the following spring. Northern ecotypes of *Populus trichocarpa*, which presumably survive colder winters than their southern counterparts, accumulate more anthocyanins than southern ecotypes with decreasing photoperiod (118). Krol *et al.* (18) believe anthocyanins protect the mesophyll of young *Pinus* seedlings from low-temperature photoinhibition. Anthocyanin-rich species such as *Photinia* have extended growing periods compared to other ornamental shrubs (119), perhaps as a result of increased tolerance of cool temperatures. A preliminary study of cold hardiness of green, UV-shielded and red, UV-exposed *Cotinus* leaves indicates that UV-exposure likewise increases the cold hardiness of this species (Chalker-Scott, unpublished data).

Any purported mechanism by which anthocyanins could enhance frost hardiness remains unclear. One hypothesis focuses on their ability to raise leaf temperature (120,121). This theory, however, has not been well documented (18,26) and requires further investigation. A more logical explanation might involve cold hardiness induction *via* osmotic control. Following a low-temperature exposure ( $5\text{--}10^{\circ}\text{C}$ ), I believe tissues will immediately show a small but significant increase in hardiness. The mechanism of this increase in frost hardiness (seen during fall and spring) is osmotic—more solutes (*e.g.* anthocyanins) in the vacuole mean water freezes at a lower temperature. This small increase in har-

diness would be enough to protect young tissues from frost damage in late spring. In particular, the accumulation of anthocyanins in epidermal vacuoles would prevent their freezing, especially from leaf surface nucleators. This phenomenon would also protect deciduous leaves from early fall frosts—a physiologically important time in which to mobilize substances for winter storage. Because this mobilization includes sugar transfer, anthocyanins might also facilitate this process because they exist almost exclusively as glycosides (112). Perennial tissues then show a second, more significant increase in cold hardiness (seen during the winter) several weeks postexposure that may or may not be related to anthocyanins.

An osmotically induced increase in cold hardiness could provide cross-resistance to other stresses, particularly drought. During winter freezes this could be particularly important in protecting sensitive parenchyma cells in the mesophyll or xylem rays of woody perennials from freeze-induced dehydration.

Apart from the problems of ice formation in leaf tissues, cold temperatures also decrease saturation levels of membrane lipids. Membranes with more polyunsaturated fatty acids are more sensitive to UVB damage because they are readily oxidized by radicals formed by UVB (122). Radicals are also longer-lived at lower temperatures, increasing the likelihood of membrane damage. Thus, epidermal anthocyanins are dually protective in preventing damage caused, directly or indirectly, by cold temperatures and UVB.

#### Environmental strain reduction—Drought resistance

Like freezing stress, drought induces water migration from cells resulting in dehydration and eventual plasmolysis. Plant cells that survive drought conditions generally have extremely thick cuticles (123) and other structural modifications. Tissues that are developmentally unable to synthesize structurally protective compounds in their cell walls (like juvenile leaves) often increase vacuolar solutes to decrease leaf osmotic potential, allowing the plant to remain turgid under low water potential conditions.

Given the induction of anthocyanins by osmotic stress, it is not surprising to find that plant tissues containing anthocyanins are often resistant to drought stress. Often the drought resistance is not causatively linked to anthocyanin content by the researchers, but such coincidental information is common in the literature. For instance, the 'Pretty Purple' cultivar of pepper is more resistant to water stress than related green cultivars (99). Ornamental shrubs with high levels of anthocyanins, such as *Cotinus* and *Photinia*, tend to be more tolerant to drought conditions (119,124–126).

More direct connections have been made both in field and laboratory settings. Decades ago anthocyanin formation in *Populus* shoots was associated with drought resistance (13). Resurrection plants, which show extraordinary tolerance for dehydration, accumulate three to four times more anthocyanins during dehydration than in their fully hydrated state (88). Deciduous tropical trees of the genus *Brachystegia* produce spring flushing leaves rich in anthocyanins 2 months prior to the rainy season; these leaves have been shown to have lower stomatal conductances than the rainy season, green leaves (21,55). Anthocyanin-containing evergreen

leaves of *Mahonia*, *Viburnum* and *Rhododendron* generally had a lower water content than their green counterparts (24). Like these perennials, maize seedlings with induced anthocyanins also show similar tolerances to drought (51).

Cross-resistance to drought can also be induced by exposure to UVB. Nogués *et al.* (111) reported that UVB exposure delayed and reduced the severity of drought stress in *Pisum sativum*. Flavonoids and anthocyanins were measured, both increasing significantly and synergistically as a function of both drought and UVB; similar results were also seen in cowpea (87). In another study of cross-resistance, Petropoulou *et al.* (127) reported that UVB alleviated drought stress in *Pinus pinea* and *Pinus halepensis*. Although anthocyanins were not specifically measured in this study, *Pinus* species are known to produce anthocyanins under UVB exposure (64).

It is interesting to note that this cross-resistance can also occur in the reverse, with the concomitant accumulation of anthocyanins. Water stress helped soybeans avoid additional stress from UVB (110,128), increasing the level of anthocyanins and other UV-absorbing compounds in leaf tissues by 23% (110). Sullivan and Teramura (128) also note that drought may reduce plant phosphorous levels, the deficiency of which had previously been linked to UVB resistance. Coincidentally, one of the classic symptoms of phosphorous deficiency is accumulation of anthocyanins in the leaves.

Drought avoidance is generally seen in plants that can decrease their leaves' osmotic potential to the point where evapotranspiration is minimized. Anthocyanin concentration will *de facto* decrease the osmotic potential (*i.e.* make it more negative) of leaves, decreasing the leaf water potential and perhaps contributing to the decreased stomatal conductance seen in flushing leaves of *Brachystegia spiciformis* (21) and drought stress resistance in *Photinia* × *fraseri* 'Red Top' (124).

#### Environmental strain reduction—Antioxidative abilities

The antioxidant capabilities of anthocyanins have only recently been investigated. Grace *et al.* (22) noticed that levels of antioxidant enzymes such as superoxide dismutase showed either slight increases or actually decreased in conjunction with photoinduced anthocyanin production in *Mahonia repens*. Ozone exposure was found to induce anthocyanins (117); it is possible that anthocyanins could have antioxidant functions in the presence of this powerful oxidizer. A similar speculation is provided by Sherwin and Farrant (88), who noted significant anthocyanin production during dehydration of resurrection plants. Ishii *et al.* (129) found the antioxidative ability of isolated anthocyanins to be greater than that of  $\alpha$ -tocopherol. Perhaps the most convincing evidence comes from Tsuda *et al.* (130,131) in their work with liposomes, microsomes and membrane systems. In all systems they noted that introduced anthocyanins scavenged oxygen radicals and inhibited lipid peroxidation.

#### Environmental strain reduction—Antifungal/antibiotic capabilities

Only speculation seems to exist on the role of anthocyanins as antifungal or antibiotic agents. Because fungus-growing leaf cutter ants avoid high levels of anthocyanins, Coley and

Aide (132) propose an antifungal role based on research by Powers (133) and others. Similarly, Tuohy and Choinski (55) hypothesize that anthocyanin-containing, spring flushing foliage of *Brachystegia* are antifungal, even though these leaves are produced prior to the wet season. Insects may be deterred by anthocyanins in tomatoes (19), but again this is speculation based on previous work (134) and has been actively discounted in other studies (26,132).

#### Cross-resistance

Many authors have commented on the similarities among the physiological and morphological responses to various abiotic stresses including UVB, cold and drought. Previous research by the author (135,136) and others (137) has demonstrated that resistance to UVB also increases cold hardiness, as does nutrient, drought and other stresses (138). Production of lignin, tannins, suberin, anthocyanins and other secondary compounds occurs in tandem with exposure to environmental stress.

While many induced cross-resistances may be due to cell wall modifications, it is more likely that developing leaves (which necessarily lack these modifications) would rely on vacuolar substances to attenuate radiation and modify water relations. Anthocyanins would seem to be good general protectors for a number of reasons:

First, anthocyanins are extremely soluble in water as they occur almost exclusively as glycosides (112) and would therefore readily accumulate in vacuoles. It is important to realize that osmotic stress can be induced by various environmental factors including radiation absorption, temperature extremes and relative water gradients (139), so resistance to these stresses is directly or indirectly dependent upon water relations within tissues.

Secondly, the fact that anthocyanins are glycosylated allows them to bind and transport reactive monosaccharides produced during developmentally or environmentally critical stages. The location of anthocyanins in ray parenchyma of cold-hardy trees (72) might very well serve in this capacity.

Thirdly, anthocyanins have the ability to attenuate UVB if appropriately acylated with hydroxycinnamic acids. Even without acylation, anthocyanins can significantly attenuate visible radiation, which might be adaptive for young leaf tissues that lack adequate structural protection to avoid photooxidation from high levels of blue light.

I believe that anthocyanins in leaf tissues have a dual function as absorbers of harmful levels and/or wavelengths of radiation and as osmotic adjusters. This second function has at least two environmentally important consequences—when the water potential of the epidermis is lowered, two environmental stresses can be avoided: ice nucleation *via* freezing events on the leaf surface and drought. Krol *et al.* (18) speculate that the phenomenon of anthocyanin development in young *Pinus* seedlings may somehow help them establish under a suite of suboptimal environmental conditions including photooxidation, low temperature, water and nutrient stress. Thus, leaf anthocyanins may be triply protective in preventing damage caused, directly or indirectly, by cold temperatures, drought and UV radiation.

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## REFERENCES

1. Dooner, H. K., T. P. Robbins and R. A. Jorgensen (1991) Genetic and developmental control of anthocyanin biosynthesis. *Annu. Rev. Genet.* **25**, 173–199.
2. Holton, T. A. and E. C. Cornish (1995) Genetics and biochemistry of anthocyanin biosynthesis. *Plant Cell* **9**, 1071–1083.
3. Mol, J., G. Jenkins, E. Schäfer and D. Weiss (1996) Signal perception, transduction, and gene expression involved in anthocyanin biosynthesis. *Crit. Rev. Plant Sci.* **15**, 525–557.
4. Mumford, P. M. (1990) Dormancy break in seeds of *Impatiens glandulifera* Royle. *New Phytol.* **115**, 171–175.
5. Kaliamoorthy, S. and A. S. Rao (1994) Effect of salinity on anthocyanin accumulation in the root of maize. *Ind. J. Plant Physiol.* **37**, 169–170.
6. Wetzal, A., M. Parniske and D. Werner (1995) Pleiotropic effect of fluoranthene on anthocyanin synthesis and nodulation of *Medicago sativa* is reversed by the plant flavone luteolin. *Bull. Environ. Contam. Toxicol.* **54**, 633–639.
7. Singh, K. B., R. S. Malhotra and M. C. Saxena (1995) Additional sources of tolerance to cold in cultivated and wild *Cicer* species. *Crop Sci.* **35**, 1491–1497.
8. Yatsuhashi, H., T. Hashimoto and S. Shimizu (1982) Ultraviolet action spectrum for anthocyanin formation in broom sorghum first internodes. *Plant Physiol.* **70**, 735–741.
9. Camm, E. L., J. McCallum, E. Leaf and M. R. Koupai-Abyazani (1993) Cold-induced purpling of *Pinus contorta* seedlings depends on previous daylength treatment. *Plant Cell Environ.* **16**, 761–764.
10. Murray, J. R. and W. P. Hackett (1991) Dihydroflavonol reductase activity in relation to differential anthocyanin accumulation in juvenile and mature phase *Hedera helix* L. *Plant Physiol.* **97**, 343–351.
11. Murray, J. R., A. G. Smith and W. P. Hackett (1994) Differential dihydroflavonol reductase transcription and anthocyanin pigmentation in the juvenile and mature phases of ivy (*Hedera helix* L.). *Planta* **194**, 102–109.
12. Dutt, S. K., A. R. Bal and A. K. Bandyopadhyay (1991) Salinity induced chemical changes in *Casuarina equisetifolia* Forst. *Egypt. J. Soil Sci.* **31**, 57–63.
13. Wettstein-Westersheim, W. and H. Minelli (1962) Breeding intersectional *Populus* hybrids. *Allg. Forstztg.* **73**, 2.
14. Leng, P., H. Itamura and H. Yamamura (1993) Freezing tolerance of several *Diospyros* species and kaki cultivars as related to anthocyanin formation. *J. Jpn. Soc. Hort. Sci.* **61**, 795–804.
15. Kakegawa, K., Y. Kaneko, E. Hattori, K. Koike and K. Takeda (1987) Cell cultures of *Centaurea cyanus* produce malonated anthocyanin in UV light. *Phytochemistry* **26**, 2261–2263.
16. Shichijo, C., T. Hamada, M. Hiraoka, C. B. Johnson and T. Hashimoto (1993) Enhancement of red-light-induced anthocyanin synthesis in sorghum first internodes by moderate low temperature given in the pre-irradiation culture period. *Planta* **191**, 238–245.
17. Tignor, M. E., F. S. Davies, W. B. Sherman and J. M. Davis (1997) Rapid freezing acclimation of *Poncirus trifoliata* seedlings exposed to 10 degrees C and long days. *HortScience* **32**, 854–857.
18. Krol, M., G. R. Gray, V. M. Hurry, G. Öquist, L. Malek and N. P. A. Huner (1995) Low-temperature stress and photoperiod affect an increased tolerance to photoinhibition in *Pinus banksiana* seedlings. *Can. J. Bot.* **73**, 1119–1127.
19. Bongue-Bartelsman, M. and D. A. Phillips (1995) Nitrogen stress regulates gene expression of enzymes in the flavonoid biosynthetic pathway of tomato. *Plant Physiol. Biochem.* **33**, 539–546.
20. Burger, J. and G. E. Edwards (1996) Photosynthetic efficiency, and photodamage by UV and visible radiation, in red versus green leaf coleus varieties. *Plant Cell Physiol.* **37**, 395–399.
21. Choinski, J. S. and J. M. Johnson (1993) Changes in photosynthesis and water status of developing leaves of *Brachystegia spiciformis* Benth. *Tree Physiol.* **13**, 17–27.
22. Grace, S., B. A. Logan, A. Keller, B. Demmig-Adams and W. Adams III (1995) Acclimation of leaf antioxidant systems to light stress. *Plant Physiol.* **108**, 36.
23. Janda, T., G. Szalai and E. Páldi (1996) Chlorophyll fluorescence and anthocyanin content in chilled maize plants after return to a non-chilling temperature under various irradiances. *Biol. Plant.* **38**, 625–627.
24. Kaku, S., M. Iwaya-Inoue and K. Toki (1992) Anthocyanin influence on water proton NMR relaxation times and water contents in leaves of evergreen woody plants during the winter. *Plant Cell Physiol.* **33**, 131–137.
25. Krizek, D. T., S. J. Britz and R. M. Mirecki (1998). Inhibitory effects of ambient levels of solar UV-A and UV-B radiation on growth of cv. New Red Fire lettuce. *Physiol. Plant.* **103**, 1–7.
26. Lee, D. W., S. Brammeier and A. P. Smith (1987) The selective advantages of anthocyanins in developing leaves of mango and cacao. *Biotropica* **19**, 40–49.
27. Lee, D. W., J. B. Lowry and B. C. Stone (1979) Abaxial anthocyanin layer in leaves of tropical rainforest plants: enhancer of light capture in deep shade. *Biotropica* **11**, 70–77.
28. Lindoo, S. J. and M. M. Caldwell (1978) Ultraviolet-B radiation-induced inhibition of leaf expansion and promotion of anthocyanin production. *Plant Physiol.* **61**, 278–282.
29. Moorthy, P. and K. Kathiresan (1997) Influence of ultraviolet-B radiation on photosynthetic and biochemical characteristics of a mangrove *Rhizophora apiculata*. *Photosynthetica* **34**, 465–471.
30. Parker, J. (1962) Relationships among cold hardiness, water-soluble protein, anthocyanins, and free sugars in *Hedera helix* L. *Plant Physiol.* **37**, 809–813.
31. Tevini, M., J. Braun and G. Pieser (1991) The protective function of the epidermal layer of rye seedlings against ultraviolet-B radiation. *Photochem. Photobiol.* **53**, 329–333.
32. Whatley, J. M. (1992) Plastid development in distinctively coloured juvenile leaves. *New Phytol.* **120**, 417–426.
33. Woodall, G. S., I. C. Dodd and G. R. Stewart (1998) Contrasting leaf development within the genus *Sycygtum*. *J. Exp. Bot.* **49**, 79–87.
34. Woodall, G. S. and G. R. Stewart (1998) Do anthocyanins play a role in UV protection of the red juvenile leaves of *Sycygtum*? *J. Exp. Bot.* **49**, 1447–1450.
35. Tevini, M. (1994) UV-B effects of terrestrial plants and aquatic organisms. *Prog. Bot.* **55**, 174–190.
36. Beggs, C. J. and E. Wellmann (1985) Analysis of light-controlled anthocyanin formation in coleoptiles of *Zea mays* L.: the role of UV-B, blue, red and far-red light. *Photochem. Photobiol.* **41**, 481–486.
37. Brandt, K., A. Giannini and B. Lercari (1995) Photomorphogenic responses to UV radiation III: a comparative study of UVB effects on anthocyanin and flavonoid accumulation in wild-type and aurea mutant of tomato (*Lycopersicon esculentum* Mill.). *Photochem. Photobiol.* **62**, 1081–1087.
38. Buchholz, G., B. Ehmann and E. Wellmann (1995) Ultraviolet light inhibition of phytochrome-induced flavonoid biosynthesis and DNA photolyase formation in mustard cotyledons (*Sinapis alba* L.). *Plant Physiol.* **108**, 227–234.
39. Christie, P. J., M. R. Alfenito and V. Walbot (1994) Impact of low temperature stress on general phenylpropanoid and anthocyanin pathways: enhancement of transcript abundance and anthocyanin pigmentation in maize seedlings. *Planta* **194**, 541–549.
40. Drumm-Herrel, H. (1984) Blue/UV light effects on anthocyanin synthesis. In *Blue Light Effects in Biological Systems* (Edited by H. Senger), pp. 375–383. Springer-Verlag, Berlin.
41. Drumm-Herrel, H. and H. Mohr (1982) Effect of blue/UV light on anthocyanin synthesis in tomato seedlings in the absence of bulk carotenoids. *Photochem. Photobiol.* **36**, 229–233.
42. Fambrini, M., C. Pugliesi, P. Vernieri, G. Guiliano and S. Baronecelli (1993) Characterization of a sunflower (*Helianthus annuus* L.) mutant, deficient in carotenoid synthesis and abscisic acid content, induced by in-vitro tissue culture. *Theor. Appl. Genet.* **87**, 65–69.
43. Gitz, D. D., L. Liu and J. W. McClure (1998) Phenolic metabolism, growth, and UV-B tolerance in phenylalanine am-

- monia-lyase-inhibited red cabbage seedlings. *Phytochemistry* **49**, 377–386.
44. Hada, M., S. Tsurumi, M. Suzuki, E. Wellmann and T. Hashimoto (1996) Involvement and non-involvement of pyrimidine dimer formation in UV-B effects on *Sorghum bicolor* Moench seedlings. *J. Plant Physiol.* **148**, 92–99.
  45. Jain, V. K. and K. N. Guruprasad (1990) Involvement of riboflavin in UV-A and white light-induced synthesis of anthocyanin in *Sorghum bicolor*. *J. Exp. Bot.* **41**, 53–58.
  46. Kubasek, W. L., B. W. Shirley, A. McKillop, H. M. Goodman, W. Briggs and F. M. Ausubel (1992) Regulation of flavonoid biosynthetic genes in germinating *Arabidopsis* seedlings. *Plant Cell* **4**, 1229–1236.
  47. Mohr, H. and H. Drumm-Herrel (1983) Coaction between phytochrome and blue/UV light in anthocyanin synthesis in seedlings. *Physiol. Plant.* **58**, 408–414.
  48. Mohr, H., H. Drumm-Herrel and R. Oelmüller (1984) Coaction of phytochrome and blue/UV light photoreceptors. In *Blue Light Effects in Biological Systems* (Edited by H. Senger), pp. 6–19. Springer-Verlag, Berlin.
  49. Oelmüller, R. and H. Mohr (1985) Mode of coaction between blue/UV light and light absorbed by phytochrome in light-mediated anthocyanin formation in the milo (*Sorghum vulgare* Pers.) seedling. *Proc. Natl. Acad. Sci. USA* **82**, 6124–6123.
  50. Reddy, V. S., K. V. Goud, R. Sharma and A. R. Reddy (1994) Ultraviolet-B-responsive anthocyanin production in a rice cultivar is associated with a specific phase of phenylalanine ammonia lyase biosynthesis. *Plant Physiol.* **105**, 1059–1066.
  51. Ronchi, A., G. Farina, F. Gozzo and C. Tonelli (1997) Effects of a triazole fungicide on maize plant metabolism: modifications of transcript abundance in resistance-related pathways. *Plant Sci.* **130**, 51–62.
  52. Toguri, T., N. Umemoto, O. Kobayashi and T. Ohtani (1993) Activation of anthocyanin synthesis genes by white light in eggplant hypocotyl tissues, and identification of an inducible P-450 cDNA. *Plant Mol. Biol.* **23**, 933–946.
  53. Wellmann, E., G. Hrazdina and H. Grisebach (1976) Induction of anthocyanin formation and of enzymes related to its biosynthesis by UV light in cell cultures of *Haplopappus gracilis*. *Phytochemistry* **15**, 913–915.
  54. Oren-Shamir, M. and A. Levi-Nissim (1997) Temperature effect on the leaf pigmentation of *Cotinus coggygria* 'Royal Purple.' *J. Hort. Sci.* **72**, 425–432.
  55. Tuohy, J. M. and J. S. Choiniski, Jr. (1990) Comparative photosynthesis in developing leaves of *Brachystegia spiciformis* Benth. *J. Exp. Bot.* **41**, 919–923.
  56. Lee, D. W. and J. B. Lowry (1980) Young-leaf anthocyanin and solar ultraviolet. *Biotropica* **12**, 75–76.
  57. Dong, Y.-H., L. Beuning, K. Davies, D. Mitra, B. Morris and A. Kootstra (1998) Expression of pigmentation genes and photo-regulation of anthocyanin biosynthesis in developing Royal Gala apple flowers. *Aust. J. Plant Physiol.* **25**, 245–252.
  58. Ambasht, N. K. and M. Agrawal (1995) Physiological responses of field grown *Zea mays* L. plants to enhanced UV-B radiation. *Biotronics* **24**, 15–23.
  59. Arakawa, O. (1988) Photoregulation of anthocyanin synthesis in apple fruit under UV-B and red light. *Plant Cell Physiol.* **29**, 1385–1389.
  60. Batschauer, A., M. Toeholl, T. Kaiser, A. Nagatani, F. Masaki and E. Schafer (1996) Blue and UV-A light regulated CHS expression in *Arabidopsis* independent of phytochrome A and phytochrome B. *Plant J.* **9**, 63–69.
  61. Gläbgen, W. E., A. Rose, J. Madlung, W. Koch, J. Gleitz and H. U. Seitz (1998) Regulation of enzymes involved in anthocyanin biosynthesis in carrot cell culture in response to treatment with ultraviolet and fungal elicitors. *Planta* **204**, 490–498.
  62. Gleitz, J., J.-P. Schnitzler, D. Steimle and H. U. Seitz (1991) Metabolic changes in carrot cells in response to simultaneous treatment with ultraviolet light and a fungal elicitor. *Planta* **184**, 362–367.
  63. Reuber, S., J. F. Bornman and G. Weissenböck (1996) Phenylpropanoid compounds in primary leaf tissues of rye (*Secale cereale*). Light response of their metabolism and the possible role in UV-B protection. *Physiol. Plant.* **97**, 160–168.
  64. Yakimchuk, R. and J. Hoddinott (1993) The influence of ultraviolet-B light and carbon dioxide enrichment on the growth and physiology of seedlings of three conifer seedlings. *Can. J. For. Res.* **24**, 1–8.
  65. Arakawa, O., Y. Hori and R. Ogata (1985) Relative effectiveness and interaction of ultraviolet-B, red and blue light in anthocyanin synthesis of apple fruit. *Physiol. Plant.* **64**, 323–327.
  66. Kerckhoffs, L. H. J., M. E. L. Schreuder, A. Van Tuinen, M. Koornneef and R. E. Kendrick (1997) Phytochrome control of anthocyanin biosynthesis in tomato seedlings: analysis using photomorphogenic mutants. *Photochem. Photobiol.* **65**, 374–381.
  67. Lin, C., M. Ahmad and A. R. Cashmore (1996) *Arabidopsis* cryptochrome 1 is a soluble protein mediating blue light-dependent regulation of plant growth and development. *Plant J.* **10**, 893–902.
  68. Takeda, J. and S. Abe (1992) Light-induced synthesis of anthocyanin in carrot cells in suspension. IV. The action spectrum. *Photochem. Photobiol.* **56**, 69–74.
  69. Graham, T. L. (1998) Flavonoid and flavonol glycoside metabolism in *Arabidopsis*. *Plant Physiol. Biochem.* **36**, 135–144.
  70. Leyva, A., J. A. Jarillo, J. Salinas and J. M. Martinez-Zapater (1995) Low temperature induces the accumulation of phenylalanine ammonia-lyase and chalcone synthase mRNAs of *Arabidopsis thaliana* in a light-dependent manner. *Plant Physiol.* **108**, 39–46.
  71. Oren-Shamir, M. and A. Levi-Nissim (1997b) UV-light effect on the leaf pigmentation of *Cotinus coggygria* 'Royal Purple.' *Sci. Hort.* **71**, 59–66.
  72. Schmucker, T. (1947) Anthocyanin im Holz der Rotbuche. *Naturwissenschaften* **34**, 91.
  73. McKown, R., G. Kuroki and G. Warren (1996) Cold responses of *Arabidopsis* mutants impaired in freezing tolerance. *J. Exp. Bot.* **47**, 1919–1925.
  74. Tholalabavi, A., J. J. Zwiazek and T. A. Thorpe (1997) Osmotically-stressed poplar cell cultures: anthocyanin accumulation, deaminase activity, and solute composition. *J. Plant Physiol.* **151**, 489–496.
  75. Tholalabavi, A., J. J. Zwiazek and R. A. Thorpe (1994) Effect of mannitol and glucose-induced osmotic stress on growth, water relations, and solute composition of cell suspension cultures of poplar (*Populus deltoides* var *occidentalis*) in relation to anthocyanin accumulation. *In Vitro Cell Dev. Biol.* **30P**, 164–170.
  76. Cormier, F., H. A. Crevier and C. B. Do (1989) Effect of sucrose concentration on the accumulation of anthocyanins in grape (*Vitis vinifera*) cell suspension. *Can. J. Bot.* **68**, 1822–1825.
  77. Decendit, A. and J. M. Merillon (1996) Condensed tannins and anthocyanin production in *Vitis vinifera* cell suspension cultures. *Plant Cell Rep.* **15**, 762–765.
  78. Do, C. B. and F. Cormier (1991a) Accumulation of peonidin 3-glucoside enhanced by osmotic stress in grape (*Vitis vinifera* L.) cell suspension. *Plant Cell Tissue Organ Cult.* **24**, 49–54.
  79. Do, C. B. and F. Cormier (1991) Effects of low nitrate and high sugar concentrations on anthocyanin content and composition of grape (*Vitis vinifera* L.) cell suspension. *Plant Cell Rep.* **9**, 500–504.
  80. Rajendran, L., G. A. Ravishankar, L. V. Venkataraman and K. R. Prathiba (1992) Anthocyanin production in callus cultures of *Daucus carota* as influence by nutrient stress and osmoticum. *Biotechnol. Lett.* **14**, 707–712.
  81. Sakamoto, K., I. Kumiko, K. Sawamura, H. Kyoko, A. Yoshihisa, Y. Takafumi and F. Tsutomu (1994) Anthocyanin production in cultured cells of *Aralia cordata* Thumb. *Plant Cell Tissue Organ Cult.* **36**, 21–26.
  82. Suzuki, M. (1995) Enhancement of anthocyanin accumulation by high osmotic stress and low pH in grape cells (*Vitis* hybrids). *J. Plant Physiol.* **147**, 152–155.
  83. Hagendoorn, M. J. M., J. L. M. Zethof, E. van Hunnik and L. H. W. van der Plas (1991) Regulation of anthocyanin and lig-

- nin synthesis in *Petunia hybrida* cell suspensions. *Plant Cell Tissue Organ Cult.* **27**, 141–147.
84. Ramanjulu, S., K. Veeranjanyulu and C. Sudhakar (1993) Physiological changes induced by NaCl in mulberry var. Mysore local. *Ind. J. Plant Physiol.* **36**, 273–275.
  85. Mita, S., N. Murano, M. Akaike and K. Nakamura (1997) Mutants of *Arabidopsis thaliana* with pleiotropic effects on the expression of the gene for beta-amylase and on the accumulation of anthocyanin that are inducible by sugars. *Plant J.* **11**, 841–851.
  86. Dubé, A., S. Charti and M. M. Laloraya (1993) Inhibition of anthocyanin synthesis and phenylalanine ammonia-lyase activity of Co<sup>2+</sup> in leaf disks of *Terminalia catappa*. *Physiol. Plant.* **88**, 237–242.
  87. Balakumar, T., V. Hani Babu Vincent and K. Paliwal (1993) On the interaction of UV-B radiation (280–315 nm) with water stress in crop plants. *Physiol. Plant.* **87**, 217–222.
  88. Sherwin, H. W. and J. M. Farrant (1998) Protection mechanisms against excess light in the resurrection plants *Craterostigma wilmsii* and *Xerophyta viscosa*. *Plant Growth Reg.* **24**, 203–210.
  89. Anderson, P. C., P. B. Lombard and M. N. Westwood (1984) Leaf conductance, growth, and survival of willow and deciduous fruit tree species under flooded soil conditions. *J. Am. Soc. Hort. Sci.* **109**, 132–138.
  90. Dedaldechamp, F., C. Uhel and J. J. Macheix (1995) Enhancement of anthocyanin synthesis and dihydroflavonol reductase (DFR) activity in response to phosphate deprivation in grape cell suspensions. *Phytochemistry* **40**, 1357–1360.
  91. Trull, M. C., M. J. Guiltinan, J. P. Lynch and J. Deikman (1997) The responses of wild-type and ABA mutant *Arabidopsis thaliana* plants to phosphorus starvation. *Plant Cell Environ.* **20**, 85–92.
  92. Liskova, D., I. Zakutna and D. Kakoniová (1989) Pigment formation in willow tissue culture. *Biol. Bratisl.* **44**, 1039–1045.
  93. Franceschi, V. R. and H. D. Grimes (1991) Induction of soybean vegetative storage proteins and anthocyanins by low-level atmospheric methyl jasmonate. *Proc. Natl. Acad. Sci. USA* **88**, 6745–6749.
  94. Ferreres, F., M. I. Gil, M. Castaner and F. A. Tomas-Barberan (1997) Phenolic metabolites in red pigmented lettuce (*Lactuca sativa*). Changes with minimal processing and cold storage. *J. Agric. Food Chem.* **45**, 4249–4254.
  95. Dixon, R. A., M. J. Harrison and C. J. Lamb (1994) Early events in the activation of plant defense responses. *Annu. Rev. Phytopathol.* **32**, 479–501.
  96. Lo, S. C. C. and R. L. Nicholson (1998) Reduction of light-induced anthocyanin accumulation in inoculated sorghum mesocotyls: implications for a compensatory role in the defense response. *Plant Physiol.* **116**, 979–989.
  97. Nemat-Alla, M. M. and M. E. Younis (1995) Herbicide effects on phenolic metabolism in maize (*Zea mays* L.) and soybean (*Glycine max* L.) seedlings. *J. Exp. Bot.* **46**, 1731–1736.
  98. Deikman, J. and P. E. Hammer (1995) Induction of anthocyanin accumulation by cytokinins in *Arabidopsis thaliana*. *Plant Physiol.* **108**, 47–57.
  99. Bahler, B. D., K. L. Steffen and M. D. Orzolek (1991) Morphological and biochemical comparison of a purple-leafed and a green-leafed pepper cultivar. *HortScience* **26**, 736.
  100. Tevini, M. (1993) Effects of enhanced UV-B radiation on terrestrial plants. In *UV-B Radiation and Ozone Depletion: Effects on Humans, Animals, Plants, Microorganisms, and Materials* (Edited by M. Tevini), pp. 125–153. Lewis Publishers, Boca Raton, FL.
  101. Caldwell, M. M. (1981) Plant response to solar ultraviolet radiation. In *Physiological Plant Ecology I. Encyclopedia of Plant Physiology New Series*, Vol. 12A (Edited by O. L. Lange, P. S. Nobel, C. B. Osmond and H. Ziegler), pp. 170–197. Springer-Verlag, Berlin.
  102. Antonelli, F., F. Bussotti, D. Grifoni, P. Grossoni, B. Mori, C. Tani and G. Zipoli (1998) Oak (*Quercus robur* L.) seedlings responses to a realistic increase in UV-B radiation under open space conditions. *Chemosphere* **36**, 4–5.
  103. Day, T. A. (1993) Relating UV-B radiation screening effectiveness of foliage to absorbing-compound concentration and anatomical characteristics in a diverse group of plants. *Oecologia* **95**, 542–550.
  104. Johanson, U., C. Gehrke, L. O. Björn, T. V. Callaghan and M. Sonesson (1995) The effects of UV-B radiation on a subarctic heath ecosystem. *Ambio* **24**, 106–111.
  105. Skaltsa, H., E. Verykokidou, C. Harvala, G. Karabourniotis and Y. Manetas (1994) UV-B protective potential and flavonoid content of leaf hairs of *Quercus ilex*. *Phytochemistry* **37**, 987–990.
  106. Teramura, A. H. (1983) Effects of ultraviolet-B radiation on the growth and yield of crop plants. *Physiol. Plant.* **58**, 415–427.
  107. Takahashi, A., K. Takeda and T. Ohnishi (1991) Light-induced anthocyanin reduces the extent of damage to DNA in UV-irradiated *Centaurea cyanus* cell in culture. *Plant Cell Physiol.* **32**, 541–547.
  108. Li, J., T.-M. Ou-Lee, R. Raba, R. G. Amundson and R. L. Last (1993) *Arabidopsis* flavonoid mutants are hypersensitive to UV-B irradiation. *Plant Cell* **5**, 171–179.
  109. Stapleton, A. E. and V. Walbot (1994) Flavonoids can protect maize DNA from the induction of ultraviolet radiation damage. *Plant Physiol.* **105**, 881–889.
  110. Murali, N. S. and A. H. Teramura (1986) Effectiveness of UV-B radiation on the growth and physiology of field-grown soybean modified by water stress. *Photochem. Photobiol.* **44**, 215–219.
  111. Noguez, S., D. J. Allen, J. I. L. Morison and N. R. Baker (1998) Ultraviolet-B radiation effects of water relations, leaf development, and photosynthesis in droughted pea plants. *Plant Physiol.* **117**, 173–181.
  112. Robinson, T. (1991) *The Organic Constituents of Higher Plants*. Cordus Press, North Amherst, MA.
  113. Burke, M. J. and C. Stushnoff (1979) Frost hardiness: a discussion of possible molecular causes of injury with particular reference to deep supercooling of water. In *Stress Physiology in Crop Plants* (Edited by H. Mussell and R. C. Staples), pp. 197–225. John Wiley and Sons, New York.
  114. Chalker-Scott, L. (1992) Disruption of an ice-nucleation barrier in cold hardy *Azalea* buds by sublethal heat stress. *Ann. Bot.* **70**, 409–418.
  115. Ishikawa, M. (1984) Deep supercooling in most tissues of wintering *Sasa senanensis* and its mechanism in leaf blade tissues. *Plant Physiol.* **75**, 196–202.
  116. Steponkus, P. L. and F. O. Lanphear (1969) The relationship of anthocyanin content to cold hardiness of *Hedera helix*. *HortScience* **4**, 55–56.
  117. Foot, J. P., S. J. M. Caporn, J. A. Lee and T. W. Ashenden (1996) The effect of long-term ozone fumigation on the growth, physiology and frost sensitivity of *Calluna vulgaris*. *New Phytol.* **133**, 503–511.
  118. Howe, G. T., W. P. Hackett, G. R. Fournier and R. E. Klevorn (1995) Photoperiodic responses of a northern and southern ecotype of black cottonwood. *Physiol. Plant.* **93**, 695–708.
  119. Knox, G. W. (1989) Water use and average growth index of five species of container grown woody landscape plants. *J. Environ. Hort.* **7**, 136–139.
  120. Es'kin, B. I. (1960) Anthocyanin and plant frost resistance. *Bot. Sci.* **130**, 58–60.
  121. Smith, A. M. (1909) On the internal temperatures of leaves in tropical insolation, with special reference to the effect of their colour on temperature; also observations on the periodicity of the appearance of young coloured leaves of trees growing in Peradina Gardens. *Ann. Roy. Bot. Gard. Peradina* **4**, 229–297.
  122. Kramcr, G. F., H. A. Norman, D. T. Krizek and R. M. Mirecki (1991) Influence of UV-B radiation on polyamines, lipid peroxidation and membrane lipids in cucumber. *Phytochemistry* **30**, 2191–2198.
  123. Curtis, J. D., N. R. Lersten and G. P. Lewis (1996) Leaf anatomy, emphasizing unusual "concertina" mesophyll cells, of two east African legumes (Caesalpiniceae, Caesalpinioideae, Leguminosae). *Ann. Bot.* **78**, 55–59.



124. Beeson, R. C. (1992) Restricting overhead irrigation to dawn limits growth in container-grown woody ornamentals. *Hort-Science* **27**, 996-999.
125. Diamantoglou, S., S. Rhizopoulou and U. Kull (1989) Energy content, storage substances, and construction and maintenance costs of Mediterranean deciduous leaves. *Oecologia* **81**, 528-533.
126. Paine, T. D., C. C. Hanlon, D. R. Pittenger, D. M. Ferrin and M. K. Malinoski (1992) Consequences of water and nitrogen management on growth and aesthetic quality of drought-tolerant woody landscape plants. *J. Environ. Hort.* **10**, 94-99.
127. Petropoulou, V., A. Kyparissis, D. Nikolopoulos and Y. Manetas (1995) Enhanced UV-B radiation alleviated the adverse effects of summer drought in two Mediterranean pines under field conditions. *Physiol. Plant.* **94**, 37-44.
128. Sullivan, J. H. and A. H. Teramura (1990) Field study of the interaction between solar ultraviolet-B radiation and drought on photosynthesis and growth in soybean. *Plant Physiol.* **92**, 141-146.
129. Ishii, G., M. Mori and Y. Umemura (1996) Antioxidative activity and food chemical properties of anthocyanins from the colored tuber flesh of potatoes. *J. Jpn. Soc. Food Sci. Technol.* **43**, 962-966.
130. Tsuda, T., K. Shiga, K. Ohshima, S. Kawakishi and T. Osawa (1996) Inhibition of lipid peroxidation and the active oxygen radical scavenging effect of anthocyanin pigments isolated from *Phaseolus vulgaris* L. *Biochem. Pharmacol.* **52**, 1033-1039.
131. Tsuda, T., M. Watanabe, K. Ohshima, S. Norinobu, S. W. Choi, S. Kawakishi and T. Osawa (1994) Antioxidative activity of the anthocyanin pigments cyanidin 3-O-beta-D-glucoside and cyanidin. *J. Agric. Food Chem.* **42**, 2407-2410.
132. Coley, P. D. and T. M. Aide (1989) Red coloration of tropical young leaves: a possible antifungal defence? *J. Trop. Ecol.* **5**, 293-300.
133. Powers, J. J. (1964) Action of anthocyanin and related compounds in bacterial cells. In *Proceedings of the 4th International Symposium of Food Microbiology*, pp. 59-75. Goteborg, Sweden.
134. Hedin, P. A., J. N. Jenkins, D. H. Collum, W. H. White and W. L. Parrott (1983) Multiple factors in cotton contributing to resistance to the tobacco budworm, *Heliothis virescens* F. In *Plant Resistance to Insects* (Edited by P. A. Hedin), pp. 347-367. American Chemical Society, Washington, DC.
135. Chalker-Scott, L. and J. D. Scott (1998) Ultraviolet-B radiation induces cold hardiness in field-tested *Rhododendron*. *Photochem. Photobiol.* **67**, 64-65S.
136. Dunning, C. A., L. Chalker-Scott and J. D. Scott (1994) Exposure to ultraviolet-B radiation increases cold hardiness in *Rhododendron*. *Physiol. Plant.* **92**, 516-520.
137. Richter, C. and J. Hoddinott (1997) UV-B effects on growth, pigments and electrolyte leakage in conifer seedlings. *Plant Physiol.* **114S**, 98.
138. Chalker-Scott, L. and L. H. Fuchigami (1989) The role of phenolic compounds in plant stress responses. In *Low Temperature Stress Physiology in Crops* (Edited by P. H. Li), pp. 67-79. CRC Press Inc., Boca Raton, FL.
139. Levitt, J. (1980) *Responses of Plants to Environmental Stresses*. Academic Press, New York.