DO ANTHOCYANINS FUNCTION AS OSMOREULATORS IN LEAF

TISSUES?

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ABSTRACT

Water stress can be induced in plant tissues, directly or indirectly, by a number of environmental conditions. Plants exposed to drought, heat, cold, wind, flooding, or saline conditions often synthesize foliar anthocyanins in response. Although previously thought to function as a UV screen, anthocyanins may instead serve to decrease leaf osmotic potential. The resulting depression of leaf water potential could increase water uptake and/or reduce transpirational losses. Combined with other anti-stress activities attributed to anthocyanins (including their solar shield and antioxidative capacities), this phenomenon may allow anthocyanin-containing leaves to tolerate suboptimal water levels. The often transitory nature of foliar anthocyanin accumulation may allow plants to respond quickly and temporarily to environmental variability rather than through more permanent anatomical or morphological modifications.

I. Introduction

Generally associated with the brilliant red, blue, and purple coloration seen in floral tissues, anthocyanins (and the closely related betacyanins) are also common foliar constituents in a diverse assortment of plants. Anthocyanins are water-soluble pigments derived from flavonoids via the shikimic acid pathway. While foliar anthocyanins may be permanent leaf components in some species, they are developmentally or environmentally transient in many others. Perhaps best known are the transitory autumnal anthocyanins associated with deciduous leaf senescence in temperate regions. At the other end of the developmental spectrum are the juvenile anthocyanins, which appear at bud break and generally disappear after leaves are fully expanded. Finally, anthocyanins can be environmentally transient, appearing and disappearing with changes in photoperiod, temperature, and other signals. The relationship between transient anthocyanins and environmental stress resistance has been recently reviewed (Chalker-Scott, 1999).

Regardless of the developmental or environmental signal responsible, anthocyanic transcience can represent a significant metabolic cost to the plant. Energy is required to modify flavononal precursors and form anthocyanins *de novo*; likewise, their degradation also requires energy. A second potential cost of anthocyanin accumulation is the resulting interference with the light reactions of photosynthesis. Because of their ability to absorb blue and reflect red

wavelengths, anthocyanins in the upper epidermis or mesophyll of leaves theoretically compete with light harvesting by chlorophyll and carotenoids. Reductions in photosynthetic rates have been noted in red-leafed varieties of *Coleus* (Burger and Edwards, 1996) and *Capsicum annuum* (Bahler *et al*., 1991), spring flushing leaves of *Brachystegia* spp. (Choinski and Johnson, 1993, Tuohy and Choinski Jr., 1990), and the red juvenile leaves of several rainforest tree species (Woodall *et al*., 1998). The competitive advantage afforded to these plants by accumulating anthocyanins must outweigh the costs associated with manufacturing and storing these compounds. This may especially be true under environmentally stressful conditions.

In this chapter the relationships between anthocyanins and abiotic environmental stressors will be explored at the whole plant level, particularly those stressors that directly or indirectly induce dehydration stress. These stressors include drought, osmotic disrupters, cold temperature, and anoxia. Evidence in the literature that coincidentally or deliberately links the presence of anthocyanins with induced resistance will be discussed. This concept of environmental resistance can then be applied to specific life history events in an attempt to explain why some anthocyanins are developmentally transient in the context of osmotic regulation.

II. Interactions Among Environmental Stressors, Plant Water Content, and Anthocyanins

A. Drought Stress

The term "drought" is generally used in reference to an abnormal reduction in available water. Certain environments characterized as arid, therefore, are not droughty unless they receive a significantly less than normal amount of rainfall. For the purposes of this chapter, the phrase "drought stress" will be used more generally to refer to any condition in which water may be a limiting factor.

1. Impact of Drought Stress upon Plant Water Relations

Lack of available water, whether or not a normal occurrence in a given environment, has the immediate effect of decreasing turgor, most especially in the leaves. As evapotranspiration exceeds water uptake, loss of turgor generally triggers stomatal closure and a decrease in photosynthetic activity. Under prolonged stress, cellular dehydration and eventually plasmolysis will occur in drought-sensitive species. Plants that survive drought conditions generally have small, thick leaves with substantial cuticles, pubescence and/or other structural modifications (Curtis *et al.,* 1996; Li *et al*., 1996). In contrast, actively growing tissues, whose cell walls are still expanding, often increase vacuolar solutes to decrease leaf osmotic potential, allowing the plant to remain turgid under low soil water potential conditions.

Though most water stress probably occurs as a result of low soil water availability, other environmental variables can induce water stress at the leaf level. Anything that causes a loss of integrity of the leaf cuticle and/or epidermis will induce increased evapotranspiration. Strong oxidizing agents, such as ozone, are able to breach these barriers and have been associated in the literature with leaf water stress (Gunthardt-Goerg *et al.*, 1993; Johnson *et al.*, 1995; Landolt *et al.*, 1994; Maier-Maercker, 1997; Sakata, 1996). Under such conditions, evapotranspiration exceeds water uptake and leaf water stress results.

2. Evidence of Anthocyanin-Mediated Drought Resistance

a. Linkages to Morphological Adaptations to Drought Both anecdotal and experimental observations have linked drought tolerance with foliar morphological characteristics such as thickened cell walls and/or cuticles, increased pubescence, and anthocyanin accumulation. Several decades ago, research on *Populus* spp. revealed positive correlations between petiole hairiness and anthocyanin content (Fritzsche and Kemmer, 1959; Kemmer and Fritzsche, 1961). Even earlier work on *Fraxinus americana* populations had determined that the southernmost ecotype exhibited both increased anthocyanin content and leaf pubescence (Wright 1944); presumably, this ecotype would be exposed to hotter, drier conditions than more northern populations. Foliar anthocyanins have

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also been correlated with pubescence in *Fragaria* spp. (Sjulin *et al*., 2000) and glaucousness of *Eucalyptus urnigera* (Thomas and Barber, 1974).

There is evidence that these adaptations to drought are more than coincidental. Recent molecular work with *Arabidopsis* mutants has linked these characteristics at the genetic level.   Shirley *et al*. (1995) studied several loci responsible for foliar anthocyanin development and discovered that one of these was also responsible for trichome development. Thickened leaves and anthocyanin increases were found in an *Arabidopsis* mutant adapted to high light conditions (Iida *et al*., 2000); high light levels are known to cause secondary drought stress. Finally, a population of *Arabidopsis* mutants with reduced cuticular waxes was found to accumulate more anthocyanins than normal (Millar *et al*., 1998), presumably as a means to maintain foliar water.

b. Linkages to Ecophysiological Adaptations to Drought Although not commonly mentioned in context with plants from arid environments, anthocyanins are nevertheless associated with water stress in a variety of studies. Perhaps the best example of anthocyanin-associated drought tolerance is that exhibited by resurrection plants. These plants, which include the genera *Craterostigma*, *Myrothamnus*, and *Xerophyta*, are able to lose most of their available water, yet remain viable for extended periods of time. During this

process, resurrection plants replace their large central vacuole with smaller vacuoles and increase their anthocyanin content three- to fourfold over normal levels (Farrant, 2000; Sherwin & Farrant, 1998).

Less dramatic but nonetheless effective examples of drought-induced anthocyanin accumulation are found in the literature. Increases in anthocyanins and/or associated flavonoids have been noted in drought-stressed *Cotinus* spp. (Oren-Shamir, personal communication), *Cucumis sativus* (Zhi-Min *et al*., 2000), *Vaccinium myrtillus* (Laura Jaakola, personal communication), *Pisum* (Allen *et al*., 1999; Balakumar *et al*., 1993), *Pinus taeda* (Heikkenen *et al*., 1986), *Malus* cv. 'Braeburn' (Mills *et al*. 1994), *Populus* spp. (Wettstein-Westersheim and Minelli, 1962), and *Quercus* spp. (Spyropoulos & Mavormmatis, 1978). These latter authors studied *Quercus* native to environments with differing levels of water availability: *Q. ilex* is found in wet evergreen sclerophyllous forests; *Q. robur* lives in typical mesophytic environments, while *Q. coccifera* is indigenous to the hottest, driest ecosystems. While all three species increased foliar anthocyanins as relative water content decreased, *Q. coccifera* accumulated the most anthocyanins and lost the least amount of water.

Plants from a variety of environments appear to utilize foliar anthocyanins to reduce evapotranspiration. Deciduous tropical trees of the genus *Brachystegia*

produce spring flushing leaves rich in anthocyanins two months prior to the rainy season; these leaves have been shown to have lower stomatal conductances than the rainy season, green leaves (Choinski and Johnson, 1993, Tuohy and Choinski Jr., 1990). Anthocyanin-containing evergreen leaves of *Mahonia, Viburnum* and *Rhododendron* generally had a lower water potential than their green counterparts (Kaku *et al*., 1992). On the other hand, seedlings of *Corylus avellana* known to be deficient in foliar anthocyanins scorch in field conditions and die (Mehlenbacher and Thompson, 1991), perhaps from excessive water loss.

Research in Doug Schemske's lab may provide linkages between floral anthocyanins and soil moisture content (Schemske and Bierzychudek, 2001). The blue-flowered morph of *Linanthus parryae* is more reproductively competitive on drier soils than the white-flowered form. Similarly, *L. parviflorus* produces both pink- and white-flowered morphs; the first is found in serpentine soils, and the second is found in sandstone deposits. Serpentine soils dry out faster than sandstone soils, so again this difference in anthocyanin content may be associated with drought tolerance. (Although these are floral, rather than foliar, anthocyanins, the phenomenon has not been correlated to pollinator attraction and therefore could be functionally similar to leaf osmotic regulators.)

This relationship between anthocyanin content and drought tolerance has been exploited, perhaps inadvertently, in the use of ornamental landscape plants. Red leaves of *Viburnum opulus* have significantly greater osmotic pressure, and presumably less evapotranspiration, than green leaves (Chalker-Scott, unpublished results). Ornamental shrubs with high levels of anthocyanins, such as *Cotinus* and *Photinia*, have been reported to be more tolerant to drought conditions (Beeson, 1992; Diamantoglou *et al.,* 1989; Knox, 1989; Paine *et al.,* 1992). *Photinia*, a genus that often exhibits juvenile reddening, was found to use water more efficiently when compared to other common ornamental species such as *Ilex*, *Rhododendron*, *Pyracantha*, and *Juniperus* (Knox, 1989). Similarly, Paine (1992) found *Photinia* to exhibit a broad tolerance for water regimes. Other red or purple cultivars, like *Capsicum annuum* 'Pretty Purple', are known to be more tolerant to water stress than green cultivars (Bahler *et al*., 1991).

c. Linkages to Ozone-Induced Drought Other conditions besides limited soil water can induce dehydration stress in plant leaf tissues. Ozone, a photochemical air pollutant, is a strong oxidizing agent with negative impacts on leaf integrity. A variety of gymnosperms and angiosperms, including *Cryptomeria japonica* (Sakata 1996), *Picea abies* (Maier-Maercker, 1997), *Pinus elliottii* var. *elliottii* (Johnson *et al*., 1995), *Betula pendula* (Gunthardt-Goerg *et al*., 1993), and *Populus* x *euramericana* (Landolt *et al*., 1994) displayed foliar symptoms such as

nonfunctional guard cells, increased leaf conductance, and cellular collapse as a result of ozone exposure. Not surprisingly, these tissues displayed secondary drought stress symptoms as leaf integrity failed. Other research has reported increased foliar anthocyanin content in *Vaccinium myrtillus* (Nygaard, 1994), *Ipomoea purpurea* (Nouchi and Odaira, 1973) ,and *Zelkova* (Nouchi and Odaira, 1973) in response to ozone exposure.   Unfortunately, these latter studies did not determine the impact of increased foliar anthocyanin on leaf water content and subsequent drought tolerance. Obviously this is an area in need of further study.

B. Osmotic Stress

Unlike drought stress, osmotic stress can occur even when environmental water availability is normal. Osmotic stress occurs when soil water contains enough dissolved solutes that transpiration within the plant is impaired. Much of the available information in the literature is found in tissue culture experiments where growth media is manipulated with osmoticum. Another body of literature investigates halophytes, which are able to tolerate extreme saline conditions. Still other information is found in mineral nutrition studies where specific elements are found at either deficient or toxic levels. All of these experimental conditions have the commonality of reducing the plant's ability to extract water from its external environment and transport it through its tissues.

1. Impact of Osmotic Stress upon Plant Water Relations

Obviously, increased external osmoticum will induce a dehydration strain on plant cells. High solute levels in the external media will pull water from plant tissues and reduce overall water content. This process can be induced in laboratory studies and observed in the field under conditions of high solute concentrations. Resistance to osmotic stress in either circumstance is generally seen in plants that respond by increasing their internal osmoticum, thereby decreasing overall water potential to the point where water loss is minimized. Foliar anthocyanin concentration will *de facto* decrease the osmotic potential (i.e. make it more negative), decreasing the leaf water potential and retaining cellular water.

2. Evidence of Anthocyanin-Mediated Osmotic Resistance

a. Sugar and Salt Stress Cell cultures are ideal experimental units for understanding the direct impact of solutes, such as sugars, on cellular anthocyanin induction. Studies with cell cultures of various species find anthocyanin accumulation resulting from osmotic stress induced by glucose (Tholakalabavi *et al.*, 1997; Tholakalabavi *et al.*, 1994), sucrose (Cormier *et al.*, 1989; Decendit and Merillon, 1996; Do and Cormier, 1991a, 1991b; Rajendran *et al.*, 1992; Sakamoto *et al.*, 1994), and mannitol (Tholakalabavi *et al.*, 1997; Tholakalabavi *et al.*, 1994; Do and Cormier, 1991a; Rajendran *et al.*, 1992; Suzuki, 1995).

Presumably, the accumulation of anthocyanins decreases osmotic potential and thereby prevents the loss of turgor.

Experiments in whole-plant systems have shown similar results. *Arabidopsis* (Mita *et al.*, 1997), *Gerbera* flowers (Amariutei *et al.*, 1995), *Hedera helix* leaves (Murray *et al.*, 1994; Murray and Hackett, 1991), *Terminalia catappa* leaves (Dubé *et al.*, 1993), and several other species (as reviewed by Weiss 2000) all accumulated anthocyanins when grown in the presence of various sugars. Sugar-induced accumulation of anthocyanins is indicated as well in work by Jeannette *et al*. (2000). In this study, heat-girdled *Zea mays* leaves accumulated anthocyanins and remained turgid. Since heat girdling was found to impair phloem, but not xylem, transport, the expected increase of foliar sugars could certainly induce the synthesis of anthocyanins.

Exposure to salts also induces anthocyanin accumulation in roots (Kaliamoorthy and Rao, 1994), stems (Dutt *et al.*, 1991), and leaves (Ramanjulu *et al.*, 1993) of various species. Kennedy and DeFilippis (1999), in comparing the salt tolerant, anthocyanic *Grevillea ilicifolia* to the salt sensitive *G. arenaria*, admit thinking that anthocyanins are "not essential for growth and survival of plants", but conclude they are acting as osmoregulators.

Of more ecological interest are the halophytes which are able to tolerate

extremely saline conditions on land and in water. Eight mangrove species, native

to marine habitats, were found to have high anthocyanins in their evergreen

foliage during the months of October and November (Oswin *et al*., 1994). Given

that these are the months preceding the monsoon season, it is likely that the

salinity is quite high and therefore so are foliar anthocyanins. Terrestrial

halophytes include *Mesembryanthemum crystallinum*, also known as ice plant.

These and other members of the Caryophyllales contain betalains instead of

anthocyanins (Vogt *et al*. 1999). These closely related pigments apparently have

the same ecophysiological function in water retention as the anthocyanins. When

subjected to drought or water stress, *M. crystallinum* increases the size of bladder

cells at the leaf tips (Cockburn *et al*., 1996), which then accumulate betalains

(Vogt *et al*., 1999).

b. Nutrient Stress Nutrient deficiencies have long been associated with

anthocyanin accumulation. The best known example is foliar reddening from

phosphorus deficiency, identified in early nutrient exclusion experiments

(McMurtrey, 1938).   Increases in foliar anthocyanins have been reported in

phosphorus-deficient *Acer saccharum* (Bernier and Brazeau, 1988), *Arabidopsis*

(Zakhleniuk *et al*., 2001), *Cunninghamia lanceolata* (Kao *et al*., 1973), *Daucus*

carota (Rajendran *et al*. 1992), *Eucalyptus grandis* (Lacey *et al*., 1966), *Ixora*

(Broschat, 2000), and *Nepenthes* (Moran and Moran, 1998). An assortment of

other nutritional deficiencies have also been associated with foliar anthocyanin

accumulation, including boron deficiency in *Eucalyptus globulus* (Dell and

Malajczuk, 1994), magnesium deficiency in *Trifolium subterraneum* (Michalk

and Huang 1992), nitrogen deficiency in *Daucus carota* (Rajendran *et al*. 1992),

Eucalyptus nitens (Close *et al*., 2001), and *Vitis vinifera* (Do and Cormier,

1991b), sulfur deficiency in *Dendranthema grandiflora* (Huang *et al*., 1997), and

zinc deficiency in *Eucalyptus marginata* (Wallace *et al*., 1986).

These strong correlations between nutritional stress and anthocyanin

accumulation can be linked to water relations and morphological leaf

characteristics.   Early studies (Fritzsche and Kemmer, 1959; Kemmer and

Fritzsche, 1961) found positive correlations between nutrient deficiency, petiole

hairiness, and anthocyanin content in *Populus* spp; these leaf characteristics have

been previously associated with drought tolerance. Mineral stressed soils were

seen to induce cuticular thickening, anthocyanin accumulation, and premature fall

senescence in *Quercus palustris* (Boyer, 1988). Phosphorus deficiency induced

water stress in *Morus alba* (Sharma, 1995) and inhibited root conductivity in

Fraxinus pennsylvanica (Andersen *et al*., 1989); presumably the latter effect

would induce water stress as well.   Feller (1996) goes as far as to suggest that the

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sclerophyllous leaves of *Rhizophora mangle* are actually an adaptation to phosphorus deficient soils rather than a response to drought.

The causal relationship behind nutritional deficiencies, anthocyanin accumulation, and water relations has not been sufficiently explored. It is likely that many of these deficiencies are either directly or indirectly associated with water uptake or water retention, leading to the development of drought-associated characteristics such as pubescence, cuticular thickening, or anthocyanin accumulation.

c. Metal Toxicity At the other end of the nutritional spectrum are stresses associated with mineral toxicities. Often, these might be essential elements in greater than needed quantities, but non-essential minerals, especially heavy metals, can also have negative effects. Anthocyanins are known to bind many heavy metals including magnesium and aluminum, which may help plants tolerate serpentine soils and other soils containing high metal concentrations. For example, the pink-flowered form of *Linanthus parviflorus* is tolerant of serpentine soils while white-flowered populations are not (Schemske and Bierzychudek, 2001). Although these are floral and not foliar anthocyanins, the function of anthocyanins in these tissues appears to be related to heavy metal tolerance rather than pollinator attraction.

Similar anthocyanin-mediated metal tolerance appears in the published literature. In an early study, lead chloride-treated seedlings of *Acer rubrum* accumulated anthocyanins in their leaves (Davis and Barnes 1973). Other experimental efforts have linked anthocyanin accumulation to exposure to heavy metals, including aluminum (Escobar-Munera, 1988), molybdenum (Hale *et al*., 2001), and calcium, cobalt, iron, manganese, vanadium, and zinc (Suvarnalatha *et al*. 1994). Conversely, interference with ethylene biosynthesis and subsequent phenolic production was thought to be the reason for cobalt to inhibit anthocyanin accumulation in *Terminalia catappa* leaf disks (Dubé *et al*. 1993).

The mechanism(s) behind heavy metal tolerance is not clearly understood. A possible phytochelating effect is described by Hale *et al*. (2001), whereby anthocyanins bind to molybdenum and are sequestered in the vacuoles of peripheral tissues. An inability to form phytochelators (including anthocyanins) was found in *Arabidopsis* mutants with hypersensitivity to cadmium exposure (Xiang *et al*. 2001). Other evidence suggests that heavy metals affect plant water relations. Aluminum exposure is known to cause drought stress in *Glycine max* (Foy *et al*., 1993; Spehar and Galwey, 1996). A drought-tolerant cultivar of *Triticum durum* was less affected by nickel exposure than a drought-sensitive cultivar (Pandolfini *et al*. 1996). Nickel exposure causes decreased water

potential and relative water content, so drought resistance is thought to enhance

nickel resistance. Given that one of these metals (aluminum) has also been linked

to increased anthocyanin production (Escobar-Munera, 1988), it is likely that

osmotic regulation by anthocyanins plays a role in tolerating heavy metal

exposure.

C. Cold Stress

At first glance, cold stress appears to have little in common with either drought or

osmotic stress. When plant tissues are subjected to rapid decreases in

temperature, the formation of intracellular ice expands and lyses cell membranes.

Such conditions, however, do not often occur in nature. Most damage caused to

plant tissues by cold temperatures in nature is instead caused by freeze-induced

dehydration, not by physical damage from ice crystals. Therefore, the stress

induced on the plant is again related to water stress, albeit indirectly.

1. Impact of Cold Temperatures upon Plant Water Relations

Under normal rates of temperature depression, extracellular water freezes first as

it is more dilute and water molecules can easily organize into the crystalline

structure necessary for ice formation. This creates a water deficit in the

extracellular spaces, which in turn pulls water from adjacent cells. The more

dilute the cell sap, the more water can be pulled away, resulting in intracellular

dehydration stress and eventual plasmolysis.   Therefore, it would be advantageous for leaf tissues exposed to frosts or freezes to maintain a lower osmotic potential via increased solute accumulation.

2. Evidence of Anthocyanin-Mediated Freezing Resistance

In an earlier review (Chalker-Scott, 1999), the possible relationship between cold hardiness and anthocyanins has been described in greater detail. Briefly, low temperature has been shown to induce anthocyanin synthesis and accumulation in the leaves of a number of species, including *Calluna vulgaris* (Foot *et al.,* 1996) , *Centaurea cyanus* (Kakegawa *et al.,* 1987), *Cornus stolonifera* (Van Huystee *et al.*, 1967), *Cotinus* (Oren-Shamir and Levi-Nissim, 1997a, 1997b), *Malus domestica* (Leng *et al.*, 2000), *Pinus* spp. (Camm *et al.*, 1993; Krol *et al.*, 1995), *Poncirus trifoliate* (Tignor *et al.*, 1997), and *Prunus persica* (Leng *et al.*, 2000). Other studies have associated more general autumnal or winter conditions with the foliar anthocyanins of *Acer* spp. (Ishikura, 1973; Ji *et al.* 1992) *Euonymous* spp. (Ishikura, 1973), *Hedera helix* (Murray *et al.*, 1994; Parker, 1962), *Mahonia repens* (Grace *et al.*, 1998), *Pinus banksiana* (Nozzolillo *et al.*, 1990), *Rhus* spp. (Ishikura, 1973), and *Terminalia catappa* (Dubé *et al.*, 1993).

Comparison of populations from distinct provenances has revealed differential anthocyanin accumulation. Northernmost populations of *Carya illinoinensis*

(Wood *et al*., 1998) and *Populus trichocarpa* (Howe *et al.,* 1995) contained

greater foliar anthocyanins than more southern populations. Likewise, *Pinus*

contorta from different localities in Norway accumulate more anthocyanins as

altitude and latitude increase (Dietrichson, 1970).   Furthermore, red- and purple-

leaved varieties of *Acer palmatum* native to northern regions lose their

pigmentation when grown in southern climes (Deal *et al*., 1990).   Given the

generally colder and shorter growing season in higher altitude or latitude

environments, it is likely that the relationship between anthocyanins and cold

hardiness is more than coincidental.

Do foliar anthocyanins actually increase cold hardiness? Much of the evidence in

the literature suggests that they do, but few researchers have asked this specific

question. Decades ago, Parker (1962) linked anthocyanin appearance and

disappearance to cold hardiness in *Hedera helix* leaves. Es'kin (1960) postulated

a protective function of foliar anthocyanins in avoiding frost damage. Grace *et al*.

(1998) noted seasonally induced anthocyanin accumulation and disappearance in

Mahonia repens. Recently, McKown *et al.* (1996) described four *Arabidopsis*

mutants deficient in freezing tolerance were unable to accumulate anthocyanins,

suggesting some commonality between anthocyanin biosynthesis and freezing

tolerance. Finally, the seasonally-induced appearance and disappearance of

anthocyanins in *Pinus banksiana* seedlings led Nozzolillo *et al*. (1990) to suggest

that anthocyanin accumulation in this species was directly associated with

exposure to low temperature rather than any other environmental factor.

3. Anthocyanic Osmoregulation and Avoidance of Freeze Damage

Young tissues are particularly sensitive to frost damage and therefore require

protection from unseasonably cold temperatures, especially those associated with

spring frosts. Expanding leaves, with their high water content and lack of

cuticular protection, can be damaged or killed by ice formation on the leaf surface

and subsequent nucleation events within tissues. The presence of increased

osmoticum within tissues would allow leaves to supercool and avoid internal

freezing events. This phenomenon was noted in expanding leaves of *Eucalyptus*

urnigera (Thomas and Barber 1974); likewise, anthocyanins were found to

accumulate in the young leaves of *Zea mays* following cold spells (Crookston

1983).

There is little in the literature that associates water relations, anthocyanins, and

cold temperatures, however.   Anthocyanin-containing leaves of winter-hardy

Mahonia, *Rhododendron*, and *Viburnum* were shown to have a lower water

potential during the winter than green leaves of the same species (Kaku *et al.*,

1992). Similarly, Tignor *et al.* (1997) found a depression of water potential

concomitant with an increase in anthocyanin content and cold hardiness

development in *Poncirus trifoliate* following low temperature exposure.   Further

studies are obviously necessary.

In an earlier review (Chalker-Scott, 1999), I hypothesized that a low temperature

exposure would induce a small but significant increase in hardiness via osmotic

regulation: more solutes (e.g. anthocyanins) in the vacuole depress the freezing

point. This small increase in hardiness 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 during which substances are mobilized

for winter storage. Perennial tissues then show a second, more significant

increase in cold hardiness (seen during the winter) several weeks post exposure,

which may or may not be related to anthocyanins.

D. Anoxic Stress

Anoxia and hypoxia are defined as an absence or deficiency of oxygen,

respectively. Anoxic or hypoxic soils are commonly found in wetland

environments, where water fills available pore spaces within the soil at the

expense of oxygen and other gases. Obligate and facultative wetland plants are

adapted to these conditions and have a variety of morphological and biochemical

modifications to avoid root anoxia. Conversely, plants that are not normally found in anoxic soils are often unable to adapt to low oxygen levels and roots become less functional. As a result, the above ground portions of the plant are subjected to an indirect water stress.

1. Impact of Anoxia upon Plant Water Relations

Anoxic soil conditions can occur as a function of high clay content, prolonged flooding, or compaction. Though root water uptake decreases, foliar transpiration continues and subsequently the above-ground portions of the plant will become dehydrated. Thus, any mechanism that helps maintain leaf water under these conditions will improve survival.

2. Evidence of Anthocyanin-Mediated Anoxia Resistance

Anthocyanins will accumulate in plants subjected to root anoxia. Young *Arbutus menziesii* grown in compacted, waterlogged clay soils developed more foliar anthocyanins than those grown in well-drained, sandy loam (Cahill and Chalker-Scott, 2000). Similarly, *Acer rubrum* seedlings grown on silt loam accumulated more anthocyanins than those grown on sandy loam (Davis and Barnes, 1973). Flooded *Malus* and *Pyrus* trees showed anthocyanin increases in their leaves (Anderson *et al*., 1984), probably in response to the secondary drought stress imposed upon leaves by depressed root function. Moreover, *Pyrus* subjected to

flooding were able avoid abscission over a period of several months (Anderson *et al*., 1984), perhaps because the foliar anthocyanins depressed osmotic potential and reduced leaf evaporation.

III. Relationships among Anthocyanins, Developmental State, and Water Relations

As reviewed to this point, there is a great deal of information on anthocyanin induction in leaf tissues in a variety of plant species under a variety of environmental conditions. Many of these environmental conditions can be linked to plant water relations. There are instances, however, of anthocyanin accumulation where water stress appears to be of less importance; juvenile leaf reddening is the principal example. In other cases, anthocyanin accumulation is associated with autumnal or winter foliage during times of the year when water uptake and transpiration are limited. Are there relationships between leaves at these different developmental stages and anthocyanin and water content?

A. Juvenile Reddening, Leaf Expansion and Osmoregulation

1. Occurrence of Phenomenon

One of the most striking phenomena associated with anthocyanins is the juvenile reddening exhibited by a variety of plant species. Young expanding leaves accumulate anthocyanins transiently, losing these pigments once they have

reached full size. Juvenile reddening is particularly noticeable in tropical evergreens and has been reported in several studies (Lee and Lowry, 1980; Nii *et al.*, 1995; Tuohy and Choinski, 1990; Whatley, 1992; Woodall *et al.*, 1998). Representatives are found in both emergent as well as understory species and include agriculturally and horticulturally important genera such as *Ficus*, *Coffea*, *Durio*, and *Mangifera*. Opler *et al.* (1980) estimated that between 20-40% of the woody species in a typical humid, tropical forest contain juvenile anthocyanins. More recently, Lee and Collins (2001) identified 179 tropical angiosperm species that exhibit juvenile reddening.

A number of plants native to other environments also exhibit juvenile anthocyanins, though the occurrence appears to be less common than that of the tropical rainforest. The temperate Aceraceae are common juvenile accumulators (Ji *et al.* 1992) as are several *Eucalyptus* species (Hillis, 1955; Thomas and Barber, 1974). Other temperate species exhibiting juvenile reddening include *Liquidambar styraciflua* (Dillenberg *et al.* 1995), *Quercus marilandica* (Choinski and Wise, 1999), and *Photinia* (Knox, 1989; Paine *et al.*, 1992). Finally, *Brachystegia*, a deciduous tree genus native to tropical savannas, accumulates anthocyanins in its young leaves (Choinski and Johnson, 1993; Tuohy and Choinski Jr., 1990).

2. Functional Roles of Juvenile Anthocyanins

The role of anthocyanins in expanding leaf and floral tissues has not been well defined. (While floral anthocyanins have been postulated to act as pollinator attractants, expanding floral tissues are not able to utilize pollinating vectors. Therefore, these pigments may play a different role during tissue expansion.) In both young leaves and flowers it is crucial to maintain high turgor to ensure optimal expansion. Turgor pressure is the force behind cell wall expansion and if a suitable gradient is not established young tissues may not achieve full expansion. Previously, Bieleski (1993) suggested that increased osmoticum in *Hemerocallis* petals was the driving force behind petal expansion. Weiss (2000) noted that flower buds are a major sink for sugar and that flowering could be delayed if photoassimilate was not available. Anthocyanins might facilitate the transport of sugars to expanding flowers since they exist almost exclusively as glycosides. This same process would occur during leaf expansion.

This latter hypothesis would explain why so many understory rainforest species exhibit juvenile reddening. While some authors have postulated a primarily photoprotective function for anthocyanins (Hoch *et al*. 2001), this theory does not hold up under the reduced light environment of tropical forests. Relative humidity is high under the canopy and water movement through subcanopy plants can be reduced. If high osmotic conditions are established in juvenile tissues –

such as the accumulation of water-soluble pigments like anthocyanins and

betacyanins – then an appropriate gradient can be established to ensure increased

water uptake, high turgor, and cell wall expansion.

Tropical species in drier environments, such as *Brachystegia* (Choinski and

Johnson, 1993; Tuohy and Choinski Jr., 1990), may also rely upon anthocyanic

osmoregulation. Given the lack of cuticle or other epidermal protection,

expanding leaf tissues are more likely than mature leaves to lose water.

Brachystegia produce anthocyanin-rich leaves during the months prior to the

rainy season; after the rains begin, the leaves turn green. The stomatal

conductance of the red, pre-rain leaves has been found to be lower than that of

green leaves (Choinski and Johsnon, 1993; Tuohy and Choinski Jr., 1990). The

ability of anthocyanins to decrease leaf osmotic potential would allow plants to

retain water more efficiently than leaves without lowered potential.

This same ability to retain water during leaf expansion would benefit temperate

zone plants. Many temperate zone species, such as *Hedera helix* (Murray and

Jackett 1991), *Photinia* (Knox, 1989; Paine *et al*., 1992), and *Quercus*

marilandica (Choinski and Wise, 1999) exhibit juvenile reddening and, in the

case of *Quercus marilandica*, also have reduced transpiration rates and stomatal

conductance (Choinski and Wise, 1999). This last bit of evidence also suggests

an osmoregulatory ability of anthocyanins in these young leaves.

Temperate zone plants not only need to maintain high turgor for leaf expansion,

but need to protect tender growing tissues from late season freezes. Unlike

mature tissues, expanding leaves cannot cold-harden by lignifying their cell walls.

Anthocyanin accumulation by epidermal cells in these latter tissues would

decrease the osmotic potential of the epidermis and delay freezing via surface

nucleators, thus protecting the leaves from late spring frosts. Evidence from a

study on *Eucalyptus urnigera* leaves supports this idea. Thomas and Barber

(1974) found that rapid temperature drops would injure expanding *E. urnigera*

leaves; however, if temperature rates fell at more natural rates, expanding leaves

would survive. This supercooling ability could be very likely due to the

anthocyanin content of the young leaves of this species and would be an obvious

benefit to temperate zone plants that leaf out when the danger of frost is still

present.

B. Autumnal Coloration, Leaf Senescence, and Frost Protection

'…the accumulation of anthocyanins in the vacuoles of leaf cells seems to

represent a kind of extravagancy without a vital function…Whereas they seem to

have no function in trees, they contribute to the autumnal feast for the human

eyes.' (Matile 2000)

While the above sentiment would be understandable (if erroneous) a hundred

years ago, it is surprising to read it in a current publication. The accumulation of

anthocyanins in temperate, senescing deciduous leaves has been intensively

studied both descriptively and experimentally. It is known that anthocyanins are

synthesized *de novo* in response to the shorter days and cooler nights of autumn.

The ability of anthocyanins to bind with otherwise reactive sugars such as glucose

makes these pigments logical candidates for carbohydrate transport during

senescence. Indeed, the presence of foliar sugars has been shown to induce

anthocyanin synthesis as discussed earlier. In some studies, such as one

performed with leaf disks of *Terminalia catappa*, this induction has been tied to

pigment accumulation during autumn senescence (Dubé *et al*. 1993). Many other

species, including members of the Aceraceae (Ishikura, 1973; Ji *et al*., 1992),

Cornus stolonifera (Feild *et al*., 2001), *Euonymus* and *Rhus* (Ishikura, 1973) also

show anthocyanin accumulation in senescing leaf tissue.

The phenomenon of autumnal reddening has been attributed to anthocyanic

photoprotection of senescing leaves (Feild *et al*., 2001; Hoch *et al*., 2001); there

is, however, an alternate hypothesis that merits exploration. It is apparent that

most, if not all, leaf tissues with autumnal anthocyanins sequester the pigments in their upper epidermal or mesophyll layers (Hoch *et al*., 2001). Additionally, anthocyanin accumulation may only occur in outer canopy leaves (Feild *et al*., 2001). The adaxial leaf surfaces, especially those in the outer canopy, are exposed to warm, high light conditions during the day and as their cuticles erode would be more likely to lose water through evapotranspiration. There is, in fact, a gradual water loss associated with leaf senescence. Anthocyanins in these adaxial tissues would depress the osmotic potential of the leaf so as to decrease water loss and support those biochemical pathways associated with programmed senescence. Furthermore, foliar anthocyanins located in upper epidermal or mesophyll layers would serve to depress the freezing point of water in these tissues, reducing the likelihood of the internal spread of ice from nucleating events on outer canopy leaf surfaces during frosty autumn nights.

C. Winter Anthocyanins, Perennial Tissues, and Cold Hardiness

In addition to autumnal frosts, temperate zone perennials undergo an annual low temperature stress which can manifest itself as freeze-induced dehydration. Above-ground tissues are especially at risk, and none more so than evergreen leaves. While dormant structures, such as buds, have various mechanisms by which to resist dehydration stress, leaves remain functional, hydrated, and susceptible to freeze damage. It is not surprising that many of these evergreen

perennials are found to contain foliar anthocyanins during the winter months. Many of these species lose their red coloration in the warmer spring and summer months (Grace *et al*., 1998; Leng *et al*., 2000; Murray *et al*., 1994; Nozzolillo *et al*., 1990; Parker, 1962), providing more evidence of the importance of these pigments during freezing conditions.

As discussed earlier, foliar anthocyanins located in upper epidermal or mesophyll layers would serve to depress the freezing point of water in these tissues, reducing the likelihood of the internal spread of ice from nucleating events on the leaf surface.   Additionally, the presence of cellular anthocyanins would decrease the solute potential and inhibit water migration into extracellular spaces. As the threat of spring frost declines, anthocyanins are catabolized and leaves return to their normal green condition (assuming that water conditions remain optimal in leaf tissues).

IV. Anthocyanic Transience and Variability in Plant Water Status

Anthocyanins often appear transiently at specific developmental stages and may be induced by a number of environmental factors, including visible and UV-B radiation, cold temperatures, drought, salinity, mineral toxicity, or nutrient stress (Chalker-Scott, 1999).   Given their potential multifunctionality, the production and localization of anthocyanins in leaf tissues may help seedlings (Krol *et al.,*

1995) or expanding tissues establish under a suite of sub-optimal environmental conditions, many of which will directly or indirectly influence plant water relations. These possible functions are summarized below.

Reduction of Evapotranspiration

Pubescence, thickened cuticles and cell walls all help decrease foliar water loss in mature tissues. Unlike these morphological modifications, however, the accumulation of anthocyanins can be transient and is therefore a more flexible adaptation. Furthermore, it is more likely that developing leaves, which lack these morphological modifications, would rely on vacuolar substances to modify water relations.

Osmotic Regulation

Anthocyanins are highly water soluble, especially as glycosides, and are usually found in vacuoles. The increased accumulation of solute in any plant cell will allow it to maintain tolerable water conditions even under high levels of external osmoticum. This has particularly important implications for plants resistant to saline habitats.

Antioxidant Behavior

Plants in suboptimal water conditions not only experience dehydration stress but

increased risk of oxidative stress from free radicals. Most, if not all,

environmental stresses occur with a concomitant production of oxygen radicals or

other reactive substances that can cause membrane damage unless attenuated by

antioxidants. Given the antioxidative properties of anthocyanins (discussed

elsewhere in this volume), their presence could protect sensitive structures such as

membranes (Leng *et al*., 2000) or chlorophyll from degradation. and also increase

the percentage of bound water within the cells. This phenomenon in the extreme

has been documented in resurrection plants.

Solar Protection

Although solar protection may not be a universal feature in all anthocyanic leaf

tissue, this attribute nonetheless would serve to diminish the possibility of

photooxidative stress, especially during high light, low temperature periods. The

role of anthocyanins in solar protection is discussed elsewhere in this volume.

Sugar Transport

Anthocyanins are generally glycosylated and therefore extremely soluble

compounds (Robinson, 1991). This capability allows anthocyanins to bind and

transport reactive monosaccharides produced during developmentally or

environmentally critical stages. In addition to their presence in senescing leaves

and expanding leaves and flowers, anthocyanins in ray parenchyma of cold hardy

trees (Schmucker, 1947) might very well serve in this capacity.

Heavy Metal Chelation

Since anthocyanins can apparently bind and sequester heavy metals, many of

which cause secondary drought stress, their presence may allow plants to survive

in metal-rich soils.

Freeze Avoidance

When the water potential of adaxial leaf surfaces is lowered, two environmental

stresses can be avoided: ice nucleation via freezing events on the leaf surface and

freeze-induced drought. During winter freezes this could be particularly

important in protecting woody evergreens from freeze-induced dehydration of

sensitive parenchyma cells both in the mesophyll and in xylem rays.

A cautionary note needs to be inserted lest one regards anthocyanins to be unique

in their transitory protective nature. Because these pigments are visible to the

human eye, they have attracted more attention than their numerous relatives in the

phenolic acid family. It may well be that less visible, and hence less studied,

chemical relatives show the same type of transience in plant tissues relative to

developmental or environmental cues.

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