Foliar Anthocyanins in Coleus and Ornamental Grasses: Accumulation, Localization, and Function

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Jennifer Kay Boldt

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John E. Erwin and Mary H. Meyer, advisers

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Dedication

This dissertation is dedicated to Mom and Dad, for initially exposing me to the wonderful world of plants.

Abstract

Anthocyanins provide red coloration in plants. The research objectives were to (1) investigate the influence of environmental factors on anthocyanin accumulation, (2) compare photosynthetic rates of red and green leaves, (3) determine anthocyanin localization in leaves, and (4) determine anthocyanin function(s) in leaves. Coleus (*Solenostemon scutellarioides*) and ornamental grasses, commonly cultivated for their foliage coloration, were selected as model plants. Irradiance and temperature influenced anthocyanin content in red coleus. Anthocyanin content increased with increasing irradiance, although photobleaching occurred in some cultivars at the highest irradiance. Exposure to low temperature (12 °C) resulted in maximum anthocyanin content in two cultivars but minimal anthocyanin content in another. In switchgrass and purple fountaingrass, anthocyanin content in individual leaves and the percent red leaves increased with increasing irradiance. Intensified seasonal leaf coloration in red-leaved grasses (*Imperata cylindrica*, *Panicum virgatum*, *Pennisetum advena*, *Pennisetum purpureum*, and *Schizachyrium scoparium*) resulted from increased anthocyanins and decreased chlorophyll. Anthocyanins were negatively correlated with average daily temperature and daily light integral (DLI) and positively correlated with total growing degree days and total DLI. Annual and non-native grasses had minimal seasonal fluctuations in pigmentation relative to native grasses (*P. virgatum* and *S. scoparium*), and this seasonal increase in anthocyanins might be an adaptive mechanism. At saturating irradiance, neither leaf color had a distinct advantage. Maximum photosynthetic rates (A_{max}) in red and green coleus were similar per area, higher in red

per fresh or dry weight, and higher in green per unit chlorophyll. A_{max} in switchgrass was higher in green leaves per area, fresh, or dry weight, and similar in red and green leaves per unit chlorophyll. Anthocyanins in coleus and switchgrass localized in epidermal cells. They were ideally situated to provide a photoprotection role as light attenuators. Anthocyanins offered minimal photoprotection in switchgrass and their presence may simply be due to selection for desired ornamental attributes. Photoprotection by anthocyanins was most evident in coleus during low temperature/high irradiance stress. Red coleus exhibited less of a decline in F_v/F_m, photosynthesis, electron transport rate, and effective quantum yield than green coleus, and F_v/F_m and photosynthetic rate recovered to pre-stress levels more quickly.

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A_{max}	Maximum photosynthetic rate
AQE	Apparent quantum efficiency (ϕ); slope of the linear portion of a light response curve; approximates the proportion of photons directed towards photochemistry
CE	Carboxylation efficiency
ETR	Electron transport rate; $[(F_m' - F_s)/F_m']fI\alpha_{leaf}$, where f = fraction of absorbed quanta used by PSII (assumed to be 0.5), I = irradiance, and α_{leaf} = leaf absorptance
F_v/F_m	Ratio of variable to maximum fluorescence in a dark-adapted leaf; maximum quantum yield; $[(F_m - F_o)/F_m]$
F_v'/F_m'	Ratio of variable to maximum fluorescence in a light-adapted leaf; $[(F_m' - F_o')/F_m']$
LCP	Light compensation point; irradiance at which net photosynthesis is zero
LSP	Light saturation point; irradiance at which a further increase in irradiance will not result in any further increase in photosynthesis
P_n	Net photosynthesis
qN	Nonphotochemical quenching; $[(F_m - F_m')/(F_m - F_o')]$
qP	Photochemical quenching; $[(F_m' - F_s)/(F_m' - F_o')]$
Θ	Convexity of a light response curve; values range between 0 and 1
Φ_{PSII}	Effective quantum yield in a light adapted leaf; $[(F_m' - F_s)/F_m' = \Delta F/F_m']$

Chapter 1

Anthocyanins: A Horticultural Review

Anthocyanins, betalains, and carotenoids provide non-green coloration in plants. In addition to aesthetic value, plant pigments play important roles in pollination, light harvesting, plant defense, protection against unfavorable environmental conditions, and human health. In leaves, anthocyanins are responsible for most red, burgundy, and purple foliage. In a small number of families, betalains provide red coloration instead of anthocyanins and the occurrence of the two pigment classes is mutually exclusive. The most common foliar anthocyanins are cyanidin glycosides. The biosynthesis and regulation of anthocyanins, their putative functions in leaves, their impact on leaf photosynthesis, and factors (biotic and abiotic) influencing their accumulation will be reviewed. The major physiological roles attributed to anthocyanins include herbivory defense, photoprotection, free radical scavenging as antioxidants, and regulation of cell osmotic potential (osmoregulation). Red leaves may have higher, equivalent, or lower photosynthetic rates than comparable green leaves, depending upon species and environmental conditions. Foliar anthocyanins may localize in epidermal and/or mesophyll cells, and their location may determine their primary function and impact on leaf photosynthetic rate. Factors attributed to anthocyanin accumulation include irradiance (both light quality and quantity), temperature, UV-B radiation, nutrient deficiency, drought, high salinity, carbohydrate status, and plant growth regulators.

Introduction

Anthocyanins, betalains, and carotenoids provide most of the non-green coloration observed in plants. They are ubiquitous and can be found in flowers, fruits, seeds, bracts, leaves, stems, roots, and tubers. These pigments, besides providing essential functions to plants, provide human health benefits and visual aesthetic appeal in natural and managed landscapes (Johnson, 2002; Stintzing and Carle, 2004). They are also used as food colorants and in the production of cosmetics and pharmaceuticals (Cazzonelli, 2011; Delgado-Vargas et al., 2000).

These three pigments contribute to the vast diversity of color present in flowers and fruits. In leaves, however, anthocyanins and betalains provide most of the non-autumnal foliage coloration. This review will briefly mention betalains and carotenoids but will focus on anthocyanins and their presence in leaves. The biosynthesis, regulation, and physiological role of anthocyanins in flowers and fruits have been previously reviewed (Forkmann, 1991; Grotewold, 2006; Holton and Cornish, 1995; Kobayashi, 2009; Lancaster and Dougall, 1992; Mol et al., 1996; Saure, 1990), but to a lesser extent in leaves and other vegetative organs (Chalker-Scott, 1999; Close and Beadle, 2003; Manetas, 2006; Steyn et al., 2002). We will review anthocyanin biosynthesis and regulation, when and where anthocyanins accumulate in leaves, the proposed physiological bases for their accumulation, their impacts on leaf photosynthesis, and factors that influence their accumulation.

Plant Pigments

Anthocyanins

Anthocyanins are secondary metabolites synthesized through the flavonoid pathway. They are responsible for most of the red, purple, and blue coloration observed in flowers and fruits. Over 635 have been isolated and identified (Anderson and Jordheim, 2006). They were first named in 1835 by Ludwig Marquart, a German botanist, from the Greek words *anthos* (flower) and *kyanos* (blue) (Lee and Gould, 2002). Anthocyanins are ubiquitous in plants and can be found in flowers, fruits, bracts, leaves, stems, roots, and tubers (Hatier and Gould, 2009) in ferns, gymnosperms, and angiosperms (Harborne, 1967; Koes et al., 1994). Their main function in reproductive organs is to attract pollinators and frugivores for flower pollination and seed dispersal. In vegetative tissues, however, their function(s) are less well understood and include mitigating light and temperature stress, reducing herbivory damage, and functioning as osmoregulators during cold, drought, or salinity stress.

Betalains

Betalains are nitrogen-containing compounds that provide red and yellow coloration in a small number of plant families. Their name is derived from *Beta vulgaris* (beet), a betalain-containing species. Betalains have a narrow distribution and are only found in nine of 11 families in the Caryophyllales (Caryopyllaceae and Molluginaceae

are the exceptions) (Delgado-Vargas et al., 2000; Tanaka et al., 2008). Of evolutionary interest is the phenomenon that anthocyanins and betalains are mutually exclusive of one another in plant species (Mabry et al., 1963; Stafford, 1994).

Both anthocyanins and betalains are initially derived from the shikimic acid pathway (anthocyanins from phenylalanine and betalains from tyrosine) (Tanaka et al., 2008). Betalain biosynthesis has been previously reviewed (Delgado-Vargas et al., 2000). Betalain-containing plants have the ability to produce other flavonoids (up to proanthocyanidins) but not anthocyanins (Clement and Mabry, 1996). This has led to the hypothesis that betalain-containing species have all the necessary components of the flavonoid biosynthetic pathway except for anthocyanidin synthase (ANS) (Grotewold, 2006; Stafford, 1994).

Although anthocyanins and betalains belong to different classes of secondary compounds and are phylogenetically separate, they do share a number of similarities: 1) both require shikimic acid as an initial precursor for biosynthesis, 2) both require final glycosylation, 3) both accumulate in vacuoles and require transporters for entry into the vacuole, 4) both have similar absorption spectra when present as aglycones, and 4) both have similar suggested functions in leaves (Ibdah et al., 2002; Stafford, 1994).

A number of taxa with horticultural significance contain betalains. Food crops within betalain-producing families include *Amaranthus* (amaranth), *Beta vulgaris* (beets and Swiss chard), *Chenopodium quinoa* (quinoa), *Opuntia* (prickly pear cactus), and *Spinacia oleracea* (spinach). The colorful attributes of some floricultural crops are also the result of betalains. Taxa from betalain-producing families include the following:

Amaranthus, Celosia, Delosperma, Dorotheanthus bellidiformis (Livingstone daisy), *Gomphrena, Mesembryanthemum crystallinum* (iceplant), *Mirabilis* (four o'clocks), *Ptilotus, Portulaca, Schlumbergera* (Christmas cactus), and cacti (e.g. *Cereus, Epiphyllum, Gymnocalycium, Lobivia, Mammillaria, Opuntia, Parodia, Pereskia, Rebutia*) flowers; *Bougainvillea glabra* bracts; *Phytolacca americana* (pokeberry) fruit; *Conophytum, Lithops* and *Portulacaria* stems; and *Alternanthera, Amaranthus, Anacampseros,* and *Iresine herbstii* leaves (Delgado-Vargas et al., 2000; Strack et al., 2003; The Plant List, 2010; To and Wang, 2006). However, *Dianthus, Gypsophila,* and *Lychnis* do not produce betalains, as they belong in Caryophyllaceae, one of two families within Caryophyllales that synthesize anthocyanins rather than betalains (Grotewold, 2006).

Carotenoids

Carotenoids are most commonly associated with yellow and orange pigmentation in plants (e.g. *Calendula, Freesia, Gerbera, Lilium, Narcissus, Rosa,* and *Tagetes* flowers, as well as sweet potatoes, carrots, and squash) but may also provide red coloration [e.g. tomatoes (lycopene) and red peppers (capsanthin and capsorbin)] (Grotewold, 2006; Tanaka et al., 2008). Carotenoids are widely distributed (bacteria, algae, fungi, and plants) and more than 600 have been isolated and identified (Delgado-Vargas et al., 2000). They are derived from isoprene and are typically C_{40} compounds. Their biosynthesis, regulation, and metabolism have previously been reviewed

(Cunningham and Gantt, 1998; Demmig-Adams et al., 1996; Sandmann, 1994; Tanaka et al., 2008).

Carotenoids have a number of important functions in plants. In flowers and fruits, carotenoids aid in pollination and seed dispersal (Cazzonelli, 2011). In leaves, they are better affiliated with photosynthesis than with pigmentation, although their unmasking in the fall following the breakdown of chlorophyll (Lee et al., 2003) provides an impressive display of autumnal coloration in some genera (e.g. *Acer*, *Betula*, *Fagus*, *Fraxinus*, *Ginko*, *Populus*, and *Ulmus*). Carotenoids are important components of antenna complexes of light harvesting complexes; they also function as antioxidants and help safely dissipate excess light energy as heat via the xanthophyll cycle (Delgado-Vargas et al., 2000). Carotenoids are also substrates for the biosynthesis of abscisic acid (ABA) (Cazzonelli, 2011).

In addition, carotenoids are important for human health and nutrition (Johnson, 2002). Epidemiological studies have shown positive associations between increased carotenoid intake and decreased cancer risk (Tanaka et al., 2012). Besides their potential role in chemoprevention (Tanaka et al., 2012), they are precursors for vitamin A biosynthesis and their consumption can help prevent blindness as well as reduce the onset and progression of macular degeneration (Taylor and Ramsay, 2005). From an economic perspective, plants containing high concentrations of carotenoids are grown and sold for use as feedstock additives to enhance animal coloration, (e.g., marigold flowers in poultry feed) or as food colorants (Tanaka et al., 2008).

Anthocyanins in Flowers and Fruits

The accumulation of anthocyanins in flowers and fruits was initially studied as a way to decipher the genetics of anthocyanin inheritance (e.g. Mendel and flower color in peas, McClintock and transposons in maize) and to elucidate the biosynthetic pathway (e.g., *Antirrhinum majus*, *Petunia* x *hybrida*, *Zea mays*) (Grotewold, 2006; Mol et al., 1998). More recently, increasing anthocyanin production in flowers and fruits or introducing novel flower colors (through classical breeding or genetic engineering) has provided economic benefits to growers, mainly through increased aesthetic quality and appeal.

Anthocyanins have been linked to a number of human health benefits, presumably due to their role as antioxidants. In addition to impacting cardiovascular health, eye health, and diabetes, anthocyanins have been reported to have anti-allergenic, anti-carcinogenic, anti-inflammatory, anti-microbial, anti-mutagenic, and anti-viral benefits (Basu et al., 2010; De Pascual-Teresa et al., 2010; Ghosh and Konishi, 2007; Hannum, 2004; Neto et al., 2008; Stintzing and Carle, 2004). As a result, increased fruit consumption and breeding for increased anthocyanin content have been recommended (Wargovich et al., 2012).

In some fruits and vegetables, anthocyanins localize in the skin or peel (e.g. apple, cranberry, grape, and potato), while in others they localize in both the skin and flesh (e.g. cherry, plum, and strawberry). Anthocyanins may also localize in vegetative organs of some vegetables and herbs, and increasing the anthocyanin content in these species

through breeding or environmental manipulation can increase their health benefits. Examples include lettuce, red cabbage, red onion, rhubarb, and purple basil.

Foliar Anthocyanins

Anthocyanins can accumulate transiently or permanently in leaves (Close and Beadle, 2003). Transient expression may be due to environmental or developmental signals (Chalker-Scott, 1999; Hatier and Gould, 2009; Mancinelli, 1983). For instance, anthocyanins may accumulate temporally in young, developing leaves (Dodd et al., 1998; Manetas et al., 2003) or in senescing leaves during autumn (Hoch et al., 2001). Biotic and abiotic stresses can cause short-term anthocyanin accumulation in leaves at any stage of development and maturity (Chalker-Scott, 1999, Lev-Yadun et al., 2004; Steyn et al., 2002). Plant physiologists theorize that the transient appearance of foliar anthocyanins occurs when the need for stress mitigation arises. Some species, however, have permanent leaf coloration (uniformly across the leaf or in some variegation pattern), which leads to the following questions: What advantage(s) does increased leaf coloration offer? What is the adaptive value? Lee and Collins (2001) postulated that since anthocyanins are present in ferns, gymnosperms, and angiosperms, their original function must have been something other than to attract pollinators and seed dispersers.

Anthocyanins are nearly geographically ubiquitous and appear in habitats ranging from the Antarctic to the tropics, from arid deserts to rainforests, and from forest understories to full sun prairies. Gould (2004) affectionately called them “the Swiss

army knife of the plant kingdom" because they seem to offer a diverse array of protection mechanisms in plants. Ougham et al. (2008) takes a more 'guarded' view on the benefits of foliar anthocyanins and suggests that there are many hypotheses for the occurrence of red foliar pigmentation but little substantive data to support them.

Localization

Anthocyanins have been observed to accumulate in all leaf layers, either in a single cellular layer or in multiple layers at a time. Cooper-Driver (2001) stated anthocyanins in vegetative tissues accumulate in the spongy mesophyll. While that may be a common occurrence in many species, they commonly accumulate in the abaxial epidermis of shade-adapted plants (Gould et al., 1995). In a survey of 463 tropical plant species, comprising 370 genera and 94 families, the most frequent locations for anthocyanins (if present) in juvenile leaves were in both the palisade and spongy mesophyll, the palisade mesophyll only, or the epidermis only. In mature leaves, the most common locations were the abaxial epidermis and spongy mesophyll (Lee and Collins, 2001). *Quintinia serrata,* a New Zealand species with polymorphic leaves, accumulates anthocyanins in all cell layers but most frequently in the palisade mesophyll, solely or in conjunction with the spongy mesophyll or epidermis (Gould et al., 2002; Neill and Gould, 1999). In *Perilla frutescens* anthocyanin biosynthesis occurs only in the adaxial epidermis (Gong et al., 1997).

The localization of anthocyanins within the leaf may be important to their putative function. Anthocyanins present in upper (adaxial) epidermal cells have a greater

potential to function as light attenuators as compared to anthocyanins located in palisade or spongy mesophyll cells. Anthocyanins present in the palisade and/or spongy mesophyll are proximally located to chloroplasts (in the same cells, but still spatially separated by the vacuole) and may be better situated to help mitigate damage due to oxygen radical formation rather than attenuate light. Gould and Quinn (1999) observed that only 16% of the species examined had anthocyanins localized in epidermal or hypodermal cells, where they would be of most benefit in blocking visible and UV-B light, and the majority were located in association with photosynthetic cells.

Classification

Six anthocyanidins (aglycones) are commonly found in plants: cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin (Gayon-Ribéreau, 1972). Cyanidin glycosides, however, appear to be the most commonly occurring anthocyanins in vegetative organs (Manetas, 2006). In surveys of anthocyanin distribution in plants, cyanidin glycosides were present in 95% of autumn leaves, 93% of juvenile leaves, and 93% of permanently red leaves, but only 80% of fruits and 50% of flowers (Lawrence et al., 1939; Price and Sturgess, 1938). In a survey of 15 species with autumnal coloration, 13 contained cyanidin glycosides and the other two contained delphinidin glycosides (Lawrence et al., 1938).

Some researchers have reported the type of anthocyanin(s) present in their plant material. In the majority of species, cyanidin-derived anthocyanins are the sole or main anthocyanin present in vegetative organs (leaves, bracts, stems, or cell suspension

cultures; Table 1.1). In only a few species were cyanidins not present in vegetative organs. Delphinidin, malvidin, and peonidin glycosides, but not pelargonidin or petunidin glycosides, have been observed (Domingues et al., 2012; Stommel et al., 2009; Timberlake and Bridle, 1982; Woodall and Stewart, 1998).

Anthocyanin Biosynthesis and Regulation

Biosynthesis

Anthocyanins, like other flavonoids, have a C_6-C_3-C_6 structure, composed of two phenolic rings (Gayon-Ribéreau, 1972) (Fig. 1.1). They are one of 13 classes of flavonoids (Delgado-Vargas et al., 2000). Flavonoids absorb ultraviolet light, but anthocyanins also absorb visible light. Maximum absorption for anthocyanins in the visible range of the spectrum is between 520 and 560 nm (Delgado-Vargas et al., 2000), depending upon the core aglycone (anthocyanidin) and the number, type, and position of hydroxyl, glycosyl, methoxyl, and acyl groups attached (Gayon-Ribéreau, 1972; Manetas, 2006; Tanaka et al., 2008).

Flavonoids are derived from the phenylpropanoid pathway, which produces both primary and secondary compounds (Davies, 2004). Phenylalanine, synthesized via the shikimic acid pathway, is converted to *trans*-cinnamic acid by phenylalanine ammonia lyase (PAL) (Fig 1.2A). *Trans*-cinnamic acid is subsequently hydroxylated to form *p*-coumaric acid, then ligated to form *p*-coumaryl-CoA (Delgado-Vargas et al., 2000).

Anthocyanin biosynthesis occurs in the cytosol (Hrazdina et al., 1978) and has been well-characterized in *Arabidopsis thaliana*, *Antirrhinum majus* (snapdragon), *Petunia* x *hybrida* (petunia), and *Zea mays* (corn) (Dooner et al., 1991; Grotewold, 2006; Holton and Cornish, 1995; Quattrocchio et al., 1993; Springob et al., 2003; Tanaka et al., 2008; Winkel-Shirley, 2001). The first committed step in flavonoid biosynthesis is the stepwise condensation of three molecules of malonyl-CoA and one molecule of *p*-coumaryl-CoA, catalyzed by chalcone synthase (CHS), to form a yellow chalcone, tetrahydroxychalcone (Fig. 1.2B). Chalcone isomerase (CHI) closes the open carbon ring, forming naringenin, a colorless molecule. This isomerization does occur spontaneously in cells, but CHI increases the rate of reaction.

Flavanone-3-hydroxylase (F3H) hydroxylates naringenin to form dihydrokaempferol (DHK), a dihydroflavonol. DHK may undergo further hydroxylation, either by F3'H (forming dihydroquercetin, DHQ) or by F3'5'H (forming dihyrdomyricetin, DHM). F3'5'H is also able to convert DHQ to DHM. This hydroxylation step is an important branch point in determining which anthocyanidins can ultimately be synthesized (pelargonidin is a derivative of DHK, while cyanidin is derived from DHQ, and delphinidin is derived from DHM). Dihydroflavonol 4-reductase (DFR) reduces a dihydroflavonol to a leucoanthocyanidin. Following oxidation and dehydration by anthocyanidin synthase (ANS or LDOX), a flavylium cation (anthocyanidin) is formed. Lastly, it is glycosylated by UDP-glucose:flavonoid 3-*O*-glucosyltransferase (UF3GT) to form an anthocyanin-3-glycoside.

Further modifications may occur before transport to the vacuole for storage. Anthocyanins may be methylated (methylated cyanidin = peonidin and methylated delphinidin = petunidin or malvidin) (Holton and Cornish, 1995), acylated with aromatic and/or aliphatic acids, or further glycosylated. It is believed that anthocyanins are tagged with glutathione in the cytosol (Mol et al., 1998) and transported into the vacuole via a glutathione *S*-transferase-like protein (Grotewold, 2006). In some species, anthocyanoplasts may also enable transport into the vacuole via vesicle-mediated mass transport (Pecket and Small, 1980; Tanaka et al., 2008).

Transcriptional Regulation

Anthocyanin biosynthesis is controlled by two sets of genes: structural and regulatory. Structural genes encode enzymes that participate directly in anthocyanin biosynthesis, and regulatory genes encode proteins that regulate structural gene expression. Regulatory genes control the intensity of anthocyanin biosynthesis, as well as the spatial and temporal arrangement of anthocyanins in cells, tissues, and organs (Holton and Cornish, 1995). Three families of regulatory proteins interact to form a complex that is able to bind to the promoter region of structural genes and regulate their transcription: R2R3 MYB proteins, basic helix-loop-helix (bHLH) proteins, and WD40 proteins (Grotewold, 2006; Tanaka et al., 2008). Anthocyanin regulatory genes often control the transcription of multiple structural genes. For example, the overexpression of *PAP1* (which encodes *PRODUCTION OF ANTHOCYANIN PIGMENTS 1*, a MYB transcription factor) in *Arabidopsis thaliana* resulted in the induction of 38 genes,

including nearly all in the anthocyanin biosynthesis pathway, and resulted in a 50-fold increase in anthocyanin content relative to wild type plants (Tohge et al., 2005).

Regulation of anthocyanin biosynthesis appears to be influenced by developmental and environmental conditions. Light is critical for biosynthesis; no foliar anthocyanin accumulation occurs in the dark (Chalker-Scott, 1999). UV-B, blue, and red light have been shown to be involved in light-induction of anthocyanin biosynthesis (Chen et al., 2006; Kubasek et al., 1992; Mancinelli, 1983; Park et al., 2007; Singh et al., 1999). In addition, Chalker-Scott (1999) speculated that *cor* genes may be responsible for cold temperature activation of anthocyanin biosynthesis.

Color Modification

Factors other than total anthocyanin content and type can influence the intensity and perceived color, including vacuolar pH, co-pigmentation, metal chelation, and the addition of glycosyl, methyl, or acyl groups (Manetas, 2006). For example, three *Euphorbia pulcherrima* cultivars with different colored bracts (red, scarlet red, and dark red) all contained the same major and minor anthocyanins (Asen, 1958).

At pH <3, anthocyanins are present as colored flavylium cations, and between pH 3 and 6 they are colorless unless stabilized by co-pigmentation (Grotewold, 2006). This is important because vacuolar pH tends to be between 4 and 6 (Cooper-Driver, 2001), although it can range between 2.7 (*Vitis vinifera* L. berries) and 7.7 (*Ipomoea tricolor* Car. petals) (Stintzing and Carle, 2004). Co-pigmentation is a phenomenon in which anthocyanins form complexes with other compounds (Boulton, 2001), including other

flavonoids, phenylpropanoids, carotenoids, organic acids, aromatic acyl groups, or metals (Grotewold, 2006).

The addition of hydroxyl groups to the B ring (Fig. 1.1) or acylation with aromatic organic acids will cause anthocyanins to appear 'bluer', while methylation and glycosylation will have a 'reddening' effect (Delgado-Vargas et al., 2000). Acylation with aliphatic acids does not affect color but increases stability (Tanaka et al., 2008). Metal chelation can also help stabilize anthocyanins, and they may form associations with Al, Fe, Cu, Sn, or borate (Boulton, 2001).

Degradation

Anthocyanin content is influenced by the rate of anthocyanin biosynthesis and degradation. Anthocyanin degradation may occur due to decreased stability or active enzymatic degradation (Oren-Shamir, 2009). Light and high temperature can influence the rate of degradation. In cranberry juice extracts the half-life ($t_{1/2}$) was approximately 50 h in light and >250 h in darkness at 40 °C, whereas at 50 °C, $t_{1/2}$ was approximately 30 h in light and 40 h in darkness (Attoe and von Elbe, 1981). Polyphenol oxidase (PPO), peroxidase, and β-glucosidase can degrade anthocyanins in juice extracts (Sakamura and Obata, 1961; Zhang et al., 2005). Oren-Shamir (2009) proposed that β-glucosidase first cleaves off the sugar moiety, followed by oxidation by PPOs or peroxidase. Peroxidase is a more likely *in planta* candidate than PPOs because it is present in the vacuole. *Brunfelsia calycina* flowers undergo active degradation, changing from purple to white

within three days of opening, and this color change was correlated with increased peroxidase activity (Vaknin et al., 2005).

Physiological Basis for Occurrence

Anthocyanins should provide some benefit when present in leaves to merit the energy required for their synthesis. Our understanding of the function(s) of anthocyanins in leaves is an "unraveling mystery" (Cooper-Driver, 2001). Hypotheses posited by entomologists, ecologists, and plant physiologists suggest a diverse array of potential functions for anthocyanins. It has been proposed that their accumulation is involved in plant defense and/or minimizing the effects of abiotic and biotic stress (Chalker-Scott, 1999; Close and Beadle, 2003; Lev-Yadun et al., 2004; Steyn et al., 2002). A commonality with both hypotheses is that the allocation of a small portion of fixed carbon towards anthocyanin production can (hopefully) minimize future, and much greater, losses of fixed carbon (either due to leaf loss from herbivory or reduced photosynthetic capacity resulting from photoinhibition).

Herbivory Defense

Anthocyanic leaves may directly (as a chemical repellant) or indirectly (as a visual signal) warn and deter herbivores (Lev-Yadun and Gould, 2009). Aposematism is the use of leaf coloration by plants to warn herbivores of their defense commitment and likely unpalatability (Gould, 2004). Since juvenile leaves do not have thickened cuticles

or lignified cell walls, they likely rely on chemical rather than mechanical defenses to deter herbivores (Manetas, 2006). Plant phenols have a bitter taste and are often present in high concentrations in young leaves (Lee and Lowry, 1980). Karageorgou and Manetas (2006) reported that red juvenile *Quercus coccifera* L. leaves (relative to green leaves) had reduced rates of herbivory. It is not clear if anthocyanins themselves deter herbivory or are correlated with increasing concentrations of other plant phenolics and visually warn of their presence. Kursar and Coley (1992) proposed that juvenile leaf reddening is a more important investment for shade rather than sun-adapted species because the potential loss of photosynthetic tissue is more detrimental to shade-adapted species. In a survey of 175 species, they observed that 33% of shade tolerant species but only 3% of gap specialists had juvenile leaf reddening.

Anthocyanins may have antifungal properties or signal the presence of antifungal compounds in leaves. Leaf cutter ants use leaf pieces to culture a fungus, their main food source, and have a tendency to select against leaves with antifungal properties. Coley and Aide (1989) observed an inverse relationship between leaf anthocyanin content and the intensity of leaf disc selection by leaf cutter ants (r = -0.48). In addition, anthocyanic oat flakes were selected against in a dose-dependent manner. No follow-up study has reported on the success (or lack thereof) of culturing this fungus on anthocyanin-containing media.

Anthocyanic leaves may also deter herbivory through camouflage. Species with leaf variegation or leaf mottling are more prevalent at the canopy floor, and they may have evolved this patterning to blend in (Givnish, 1990). Dominy et al. (2002) noted that

the percentage of species with delayed greening in juvenile leaves did not differ between sun and shade environments and suggested that camouflage was a plausible explanation for the presence of anthocyanins in young leaves. Additionally, anthocyanic leaves with uniform pigmentation across the leaf surface will appear a dull brown to insects and animals and may blend into their surroundings by mimicking dead leaves (Lev-Yadun and Gould, 2009).

Lastly, rather than blending in, some anthocyanic leaves may deter herbivores by undermining insect crypsis. Green insects avoid predation by blending in with leaves, but green insects on red leaves are much more visible to their predators (Manetas, 2006). It has been suggested that green insects tend to avoid anthocyanic leaves, resulting in reduced herbivory rates (Lev-Yadun et al., 2004). Lev-Yadun and Gould (2009) summarized this hypothesis as "the enemy of my enemy is my friend".

Photoprotection

Abiotic stresses (high light, ultraviolet radiation, low temperature, nutrient deficiency, and drought) can have a negative impact on photosynthesis. Photoinhibition is defined as a reduction in photosynthesis that occurs when more photons of light are absorbed than can be directed towards photochemistry (Krause, 1988). It may be dynamic, chronic, or sustained, depending upon the length of time required to regain photosynthetic efficiency (Lambers et al., 2006). While photoinhibition resulting from damage to PSII is an irreversible process, photoinhibition as a result of increased thermal energy dissipation is a reversible process (Demmig-Adams and Adams, 1992).

Therefore, it is important to determine the underlying cause of decreased photosynthetic efficiency; increased thermal dissipation is a photoprotective mechanism and not the result of photodamage (Demmig-Adams and Adams, 1992). As such, photoinhibition is most often used to refer to instances in which damage to PSII occurs (Demmig-Adams and Adams, 1992).

Photoinhibition may occur as a result of high irradiance, bottlenecks in carbon fixation, or damage to the thylakoid membrane (Krause, 1988). Under high light, more photons are absorbed by the leaf than can safely be directed towards photochemistry due to limitations in the rate of carbon fixation or the size of the carbon fixation enzyme pool. Ultraviolet radiation can damage DNA, proteins and membranes and can also cause oxyradicals to form, which can damage thylakoid membranes if not suppressed. Low temperatures slow down enzyme activity, resulting in slower rates of carbon fixation, an excess pool of reduced cofactors, and a shortage of oxidized electron acceptors. The effects of low temperature stress are especially detrimental in conjunction with high irradiance (Krause, 1988). Phosphorus deficiency limits the production of ATP and the number of phosphorylated substrates available for carbon fixation (Havlin et al., 2005; Manetas, 2006). The commonality across all these abiotic stresses is that excess light energy needs to be safely shuttled away from photosystem II (PSII) until an equilibrium between light capture and carbon fixation re-occurs.

Plants have evolved a number of tolerance, repair, and avoidance mechanism to effectively minimize photoinhibition. These include releasing excess light energy as heat via the xanthophyll cycle, utilizing alternative electron sinks, increasing photorespiration,

reorienting leaves and chloroplasts to minimize light capture, increasing the size of the Calvin Cycle enzyme pool, increasing the size of the antioxidant pool, altering the chlorophyll *a*/*b* ratio, and decreasing chloroplast number (Demmig-Adams and Adams, 1992; Krause, 1988; Lambers et al., 2008; Steyn et el., 2002; Vuleta et al., 2011). In addition to these mechanisms, anthocyanin accumulation may help a plant acclimate and/or adapt to abiotic stress.

Plant physiologists hypothesize that anthocyanins act as a light screen and/or as free radical scavengers (Manetas, 2006; Neill and Gould, 2003). *In vivo*, anthocyanic leaves absorb more light between 450 and 600 nm than acyanic (green) leaves of similar age and leaf anatomy (Merzylak et al., 2008). Their ability to intercept green light and some blue light can help alleviate excess photon absorption by light harvesting complexes in chloroplasts.

Anthocyanins have been observed to help mitigate the effects of abiotic stress in juvenile leaves during chlorophyll formation (Karageorgou and Manetas, 2006) and in senescing leaves during chloroplast dismantling (Archetti, 2009; Feild et al., 2001; Hoch et al., 2003; Lee et al., 2003). While carotenoids are unmasked in autumn, it appears that anthocyanins are primarily synthesized *de novo* in autumn in conjunction with chlorophyll degradation (Lee et al., 2003). Their ability to shield chloroplasts during leaf senescence may help with nutrient resorption in deciduous species. For example, in *Cornus sericea*, *Vaccinium elliottii*, and *Viburnum sargentii*, three species with anthocyanin-containing and anthocyanin-deficient autumnal phenotypes, anthocyaninic leaves had higher nutrient resorption efficiencies than acyanic leaves (Hoch et al., 2003).

Similar to autumnal leaf coloration, the onset of anthocyanin accumulation in *Euphorbia pulcherrima* bracts coincided with a decline in chlorophyll (Kannangara and Hansson, 1998).

By attenuating light, anthocyanins can help decrease the frequency and severity of photoinhibitory events and allow plants to more quickly recover to pre-stress photosynthetic rates (Gould, 2004). F_v/F_m, a ratio of variable to maximum fluorescence, is one measurement commonly used to compare leaves before, during, and after a 'stress' event. It is referred to as the maximum quantum yield in a dark-adapted leaf and is approximately 0.83 in healthy, unstressed leaves, although it can vary with species (Maxwell and Johnson, 2000). The F_v/F_m ratio will decline following exposure to environmental stress (e.g., drought, low temperatures, high irradiance) if damage to PSII occurs.

Anthocyanins have been shown to reduce a decline in F_v/F_m in *Bauhinia variegata* pods (Smillie and Hetherington, 1999), *Cornus stolonifera* leaves (Feild et al., 2001), and *Zea mays* leaves (Pietrini et al., 2002) following exposure to high-intensity white but not red light, wavelengths at which they do not effectively absorb (Steyn et al., 2002). In *Cornus stolonifera*, red and green stems had similar decreases in F_v/F_m following a 30 min exposure to high light (1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PAR*), but green stems had a greater decline in F_v/F_m than red stems after a 3 h exposure (Gould et al., 2010). Red *Solenostemon scutellarioides* (coleus) leaves had less of a decline in F_v/F_m (19% decrease) than green coleus leaves (78% decrease) following a 4 d exposure to 10 °C and 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PAR* (Boldt, 2013). In addition, F_v/F_m in red coleus recovered to pre-

stress values after 1 d at ambient conditions, whereas green leaves required a 3 d recovery duration.

The ability of anthocyanins to attenuate light would be an especially significant coping mechanism if they could effectively protect against photodamage without reducing photosynthetic capacity during periods of minimal abiotic stress. Many species with red leaves tend to have lower photosynthetic rates (Barker et al., 1997; Gould et al., 2002; Tuohy and Choinski, 1990; Woodall et al., 1998; Zhang et al., 2011), although higher or similar photosynthetic rates have been observed in some red phenotypes compared to corresponding green phenotypes (Burger and Edwards, 1996; Gould et al., 1995; Marini, 1986; Pietrini et al., 2002).

One compromise between optimal light capture under non-photoinhibitory conditions and photoprotection during periods of stress may be the occurrence of transient anthocyanin accumulation. In species with permanently pigmented leaves, the presence of anthocyanins may be an adaptive mechanism for inhabiting high-stress environments (provide a constitutive, baseline level of anthocyanins at all times, with further accumulation when additional stresses occur).

Antioxidant

In addition to attenuating light, anthocyanins may help ameliorate the effects of abiotic stress by quenching excess oxygen radicals formed during these periods of stress. Neill and Gould (2003) observed a 37% reduction in chlorophyll bleaching and a 33% reduction in superoxide ($O_2^{\cdot -}$) generation in irradiated red *Lactuca sativa* L. (lettuce)

leaves as compared to green leaves, indicating that anthocyanins have the capacity to simultaneously reduce photoinhibition and the generation of oxyradicals. The antioxidant capacity of four red *Brassica oleracea* var. *capitata* cultivars, on average, was 10-fold higher than in four green cultivars, based on a FRAP (ferric reducing-antioxidant power) assay (Yuan et al., 2009). Anthocyanin content had a linear relationship with antioxidant activity (r^2 = 0.32) in *Galax urceolata* leaves (Hughes et al., 2005). It is unknown, however, if these increases in antioxidant capacity can be attributed solely to an increase in anthocyanin content or to upregulation of the entire flavonoid biosynthetic pathway.

Anthocyanins often accumulate in leaves following wounding or pathogen attack (Close and Beadle, 2003; Gould, 2004; Hatier and Gould, 2009). Cell walls pierced during wounding or degraded by pectolytic enzymes will release H_2O_2 and other reactive oxygen species (ROS) (Taiz and Zeiger, 2002). Yamasaki (1997) reported that a cyanidin glycoside was effective at quenching H_2O_2 in solution and proposed that anthocyanins could be effective oxyradical scavengers in cells since H_2O_2 is able to diffuse across cell membranes and into vacuoles, where anthocyanins reside. Neill and Gould (2003) noted that anthocyanins may also be able to function as antioxidants while still in the cytosol (before transport and storage in the vacuole). Anthocyanins that accumulate in the neighboring cells surrounding an injury site may scavenge oxyradicals in an attempt to limit their spread and minimize their impact on healthy cells nearby.

Can anthocyanins effectively function as antioxidants? In an antioxidant assay, Wang et al. (1997) reported that when anthocyanins and flavones with similar

hydroxylation patterns were compared, flavones were stronger antioxidants but anthocyanins were still capable of performing satisfactorily as antioxidants. All 14 anthocyanins examined were stronger antioxidants than Trolox, a vitamin E analog (1.07 to 3.49-fold higher), and cyanidin-3-glucoside had the highest ORAC (oxygen radical absorbance capacity) of the anthocyanins assayed. This might be a possible answer to Manetas' (2006) queries as to why are most anthocyanic leaves red and why are cyanidin glycosides the most commonly-occurring anthocyanins in leaves.

Osmoregulator

One mechanism to increase protection against cold and/or drought stress is to increase the cytosolic solute concentration (lower the osmotic potential), delaying freezing and dessication. Anthocyanins often accumulate transiently in response to cold temperatures or drought (Chalker-Scott, 2002) and may function as a compatible solute (Chalker-Scott, 1999). In a survey of tropical weed species, Veeranjaneyulu and Das (1984) noted increased in leaf pigmentation when plants were grown in soils with low moisture content.

Although anthocyanins are often correlated with increased cold tolerance, Steponkus and Lanphear (1969) found no direct causal relationship between anthocyanin content and cold tolerance in *Hedera helix* leaves. Anthocyanin accumulation in *Poncirus trifoliata* leaves and stems occurred following prolonged exposure (7 and 21 d, respectively) to 10 °C and 450 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PAR*, but their presence did not increase freezing tolerance (Tignor et al., 1997). In addition, two *A. thaliana* mutants (*ttg* and *tt4*)

that do not accumulate anthocyanins had similar levels of freezing tolerance as wild-type plants (Leyva et al., 1995). Therefore, it is likely that anthocyanin accumulation occurs as a result of cold temperature exposure but is not an acclimation or adaptation mechanism to help plants increase their tolerance to cold temperature.

Manetas (2006) suggested that anthocyanins do not accumulate in high enough concentrations to affect solute potential and therefore, do not function as osmoregulators. He suggests a well-watered mesophyte has a solute concentration around 300 $mmol \cdot kg^{-1}$ (and drought can increase this two to four-fold). However, anthocyanins only accumulate to concentrations of 1-4 $mmol \cdot kg^{-1}$ in cells, not enough to substantially alter the solute concentration.

Increase Leaf Temperature

It has been observed that red leaves were warmer than green leaves. Haberlandt (1965) reported that in the 1800s Stahl tested the hypothesis that anthocyanins increased leaf temperature. After placing leaves from red and green phenotypes equidistant from a flame, he noted that red leaves were 0.22-1.82 °C warmer than the green leaves (Haberlandt, 1965). Archetti (2009), reviewing hypotheses for autumn coloration, suggested that anthocyanins may help warm leaves in fall, but no evidence supports this. Lee et al. (1979) did not find any detectable differences in leaf temperature between red and green leaves, on either adaxial or abaxial leaf surfaces.

Specialized Functions

The following, possibly species-specific, functions have also been attributed to anthocyanins:

1. *Heavy metal sequestration* – Anthocyanins help sequester molybdenum and tungsten in *Brassica* species (Hale et al., 2001, 2002). It is not known if they facilitate uptake or storage of these metals.
2. *Protect photolabile compounds from degradation* – Page and Towers (2002) reported anthocyanins act as a light screen in *Ambrosia chamissonis* (Less.) Greene stems and petioles to inhibit the breakdown of thiarubrines (antifungal and antibacterial compounds). A 94% decrease in thiarubrine A was observed in roots (lacking anthocyanins) following a 30 min exposure to 2.3 $mW \cdot cm^{-2}$ irradiance, but thiarubrine A levels were unaffected in leaves and stems after a 4 h irradiation.
3. *Light backscattering* – This hypothesis attempted to explain why many shade-adapted plants contain anthocyanins only in the abaxial epidermis (Lee et al., 1979). It was proposed that anthocyanins increased photosynthetic rates by scattering light back into the mesophyll layer. This hypothesis was later discounted by Lee and Graham (1986).

Anthocyanins Affect Photosynthetic Rate

Maximum absorption for anthocyanins occurs in the green region of the visible spectrum, but they also absorb some blue light. This overlaps slightly with the absorbance spectrum of chlorophyll *b*, which will reduce the number of photons intercepted by PSII if anthocyanins are located above chloroplasts (Manetas, 2006). Blue and red light are the primary wavelengths used by photosynthetic cells in the upper portion of the leaf, but green light can be an important driver of photosynthesis in lower leaf layers (e.g. spongy mesophyll) (Sun et al., 1998). Therefore, the location of anthocyanins in a leaf will likely influence its photosynthetic capacity. When present in the upper epidermis, anthocyanins will attenuate light and leaves may have lower photosynthetic rates under non-saturating irradiance as compared to an identical acyanic leaf. Under stressed conditions, however, their ability to shield the chloroplasts from excess light energy may be favorable and limit photoinhibition, resulting in comparable or higher photosynthetic rates than green leaves. For example, wild-type (green) and *Lc*-transformed (red) *Petunia* x *hybrida* leaves had similar light saturation points (LSP) under low light (80 $\mu mol \cdot m^{-2} \cdot s^{-1}$), but *Lc* plants had a 46% higher LSP relative to Mitchell wild-type plants under high light (600 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (Albert et al., 2009). Young red leaves of *Quercus coccifera* exposed to 1600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ irradiance for 1 h were less prone to photoinhibition than young green leaves and had a higher F_v/F_m throughout the subsequent 19 h dark recovery period (Manetas et al., 2003).

When present in the palisade or spongy mesophyll, the ability of anthocyanins to shield chloroplasts from excess light will depend upon their localization in relation to

photosynthetic cells. As such, photosynthetic rates may be comparable to green leaves under non-stressed conditions (and potentially higher under stressed conditions if they indeed function as free radical scavengers and help mitigate damage to photosystems during periods of excess irradiance). Anthocyanins present in the abaxial surface will not block light absorption by photosynthetic cells and should not have as much of an effect on photosynthetic rates relative to comparable green leaves.

The effects of leaf coloration on photosynthesis are varied, which may be due to species differences, leaf chlorophyll content, the location of anthocyanins in the leaves sampled, or the degree of stress to which plants were exposed to prior to collection of photosynthetic measurements. Red leaves of *Begonia pavonina* and *Triolena hirsuta* had higher maximum rates of photosynthesis than green leaves (Gould et al., 1995), but they also had higher amounts of total chlorophyll, which may account for the increased photosynthetic rate per unit area. Purple *Capsicum annuum* leaves also had higher light-saturated rates of photosynthesis and chlorophyll content than green leaves (Bahler et al., 1991).

Young purple and green leaves of *Coffea arabica*, however, had similar rates of photosynthesis when measured in the morning and again at mid-day (0900 and 1300 $_{HR}$) (Domingues et al., 2012). *Prunus persica* L. (peach) leaves harvested from red and green phenotypes (derived from the same parental cross) had similar photosynthetic rates, although the green leaves contained more chlorophyll (Marini, 1986). Similar quantum yields were observed in red and green *Coleus blumei* (coleus) leaves under red or white light but were lower in red leaves under green light (Burger and Edwards, 1996).

Likewise, in a survey of nine coleus cultivars, similar rates of maximum photosynthesis per unit area were observed between red and green coleus leaves at saturating irradiance (blue and red light) (Boldt, unpublished data). However, red coleus leaves had lower maximum photosynthetic rates when it was expressed on a per unit chlorophyll basis rather than on a per unit area basis.

Lower photosynthetic rates at saturating irradiance have been observed for red leaves relative to green leaves. For example, lower rates of photosynthesis were observed in red *Panicum virgatum* (switchgrass) leaves compared to green switchgrass leaves per unit area, although they were similar when compared on a per unit chlorophyll basis (Boldt, unpublished data). Red *Quintinia serrata* leaves had a 23% lower rate of CO_2 assimilation relative to green leaves at saturating irradiance but similar rates of CO_2 assimilation at low irradiance (<100 μmol m^{-2} s^{-1}) (Gould et al., 2002). Also, red *Q. serrata* leaves light-saturated at a lower irradiance than green *Q. serrata* leaves.

Environmental Factors and Anthocyanin Accumulation

Irradiance

Light Quality

Light is critical for anthocyanin biosynthesis in leaves; no accumulation occurs in the dark (Ahmed et al., 2009; Brandt et al., 1995; Chalker-Scott, 1999). It appears that UV-B, blue, and red light are involved in light-induction of anthocyanin biosynthesis. Action peaks have been observed for UV-A (320-400 nm), UV-B (290-320 nm), blue

(400-480 nm), red (600-690 nm), and far red (710-760 nm) light, but not all are effective in every plant system (Mancinelli, 1983).

Gene expression of anthocyanin biosynthesis genes (*PAL1*, *CHS*, *CHI*, and *DFR*) in dark-grown *Arabidopsis thaliana* seedlings was induced by UV-B and blue light (Kubasek et al., 1992). Blue light induced a significant increase in anthocyanins in dark-grown *A. thaliana* seedlings at fluence rates of 6 and 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Chen et al., 2006). In addition, *A. thaliana hy4* mutants which lack cryptochrome, a blue photoreceptor, did not accumulate anthocyanins (Chen et al., 2006; Graham, 1998), while plants overexpressing *CRY1* (*cryptochrome 1*), and therefore with increased levels of chryptrochrome, had increased anthocyanin content.

Anthocyanin content in *Lactuca sativa* also appears to be regulated by UV and blue light. Following 10 d of supplemental UV-B-irradiation, *CHS*, *F3H*, and *DFR* expression in lettuce leaves were upregulated (Park et al., 2007). Anthocyanin content increased 11% and 31% in lettuce 'Red Cross' leaves exposed to supplemental UV-A and blue light, respectively, but was unaffected by supplemental green or red light, and decreased 40% in leaves grown under supplemental far red light (Li and Kubota, 2009).

In other species, anthocyanin accumulation was induced in dark-grown *Lycopersicon esculentum* Mill. hypocotyls following exposure to UV light (Brandt et al., 1995), was induced in sorghum internodes by UV and red light (Shichijo et al., 1993), and was regulated by UV-B and red light in *Zea mays* (Singh et al., 1999). Anthocyanin content in *Perilla* was higher in leaves grown under red light (Nishimura et al., 2009).

Light Quantity

Anthocyanins have been observed to accumulate in response to elevated irradiance, and they are presumed to help minimize the damaging effects of excess light on photosystems. *Petunia* x *hybrida* plants normally have green leaves but develop anthocyanin pigmentation in veins of older leaves under high irradiance (705 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (Albert et al., 2009). Nine red *Solenostemon scutellarioides* (coleus) cultivars had increased anthocyanin content as irradiance increased from 75 to 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Boldt et al., 2011a). Three *Lactuca sativa* cultivars had increased anthocyanin content as irradiance increased from 8.6 to 17.2 $mol \cdot m^{-2} \cdot d^{-1}$ (Richards et al., 2004). In another study, *L. sativa* cultivars had decreased levels of anthocyanins when grown under 50% shade (Kleinhenz et al., 2003). *Ipomoea batatas* (sweetpotato) plants grown under 0%, 40%, or 80% shading for 7 d had decreased anthocyanin content as the percent shading increased (Islam et al., 2005). *Iris pumila* L. leaves exposed to full sun had more than twice the anthocyanin content as those exposed to 65% shading (Tucić et al., 2009; Vuleta et al., 2011). Anthocyanin content in winter leaves of *Galax urceolata* increased linearly in response to light intensity (r^2 = 0.76 and 0.84 for plants grown in pots and field plots, respectively), and was 23-fold higher in full-sun plants relative to those grown under 80% shade (Hughes et al., 2005).

Capsicum annuum (pepper) grown under high irradiance (435 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for 3 to 4 months accumulated more anthocyanins than those grown under low irradiance (215 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (Lightbourn et al., 2007). Beckwith et al. (2004) sampled *Pennisetum setaceum* 'Rubrum' (purple fountaingrass) leaves from the top, middle, and lower layers

of plant canopies of greenhouse-grown plants and found that anthocyanin content decreased from top to bottom, presumably due to the effect of increased canopy shading. In a follow-up experiment in growth chambers, anthocyanin content in 'Rubum' leaves increased as the daily light integral (DLI, $mol \cdot m^{-2} \cdot d^{-1}$) increased (Beckwith et al., 2004). Likewise, Boldt et al. (2011a) observed that purple fountaingrass and *Panicum virgatum* (switchgrass) plants had an increase in both anthocyanin content and the percent of red leaves as irradiance increased.

The observed increases in anthocyanin content as a result of increased irradiance may be the result of increased rates of transcription of structural and/or regulatory genes. Lightbourn et al. (2007) observed increased expression of structural genes (*CHS*, *DFR*, and *ANS*) in *Capsicum annuum* plants grown under higher irradiance, and Gong et al. (1997) observed increased mRNA transcripts for structural genes (*F3H*, *DFR*, *3GT*, *LDOX*, and *anthocyanin acyltransferase*) in *Perilla frutescens* leaves grown under high irradiance. *Medicago sativa* (alfalfa) and *Petunia* x *hybrida* plants transformed with *Lc*, a regulatory gene from *Zea mays*, exhibited green leaves under low irradiance but red leaves under high irradiance (Albert et al., 2009; Ray et al., 2003). The high light exposure resulted in increased transcript abundance of *CHS*, *CHI*, *FLS*, *DFR*, and *ANS* in *P.* x *hybrida* and increased transcript abundance of *CHS* in *M. sativa*.

Excessive irradiance, however, can sometimes have a deleterious effect on anthocyanin content. Anthocyanins accumulated in suspension cultures of *Perilla frutescens* as irradiance increased from 0 $W \cdot m^{-2}$ to 27.2 $W \cdot m^{-2}$, but very little anthocyanin was observed in cultures exposed to 54.4 $W \cdot m^{-2}$ (Zhong et al., 1991).

Temperature may influence anthocyanin accumulation in response to irradiance. In *Cichorium intybus* L., at 15/10 and 20/15 °C day/night temperatures, anthocyanin content increased as irradiance increased (from 50 to 340 $\mu mol \cdot m^{-2} \cdot s^{-1}$), but at 30/25 °C, anthocyanin content did not differ amongst irradiance levels (Boo et al., 1997).

UV-B radiation

UV-B radiation (280-320 nm) can damage DNA, proteins, and membranes, and can be especially damaging to PSII (Jansen et al., 1998; Teramura and Sullivan, 1994). Flavonoids, including anthocyanins, have been shown to accumulate in response to UV-B exposure, especially in epidermal cells (Stapleton, 1992). Exposure to increasing fluence rates of UV-B radiation resulted in increased percent dimer formation (an indication of DNA damage) in cell suspension cultures of *Centaurea cyanus* L. (Takahashi et al., 1991). However, at each fluence rate, anthocyanic cells had a lower percent dimer formation than non-colored cells.

Using films to exclude the transmission of different UV bands, Krizek et al. (1998) and Tsormpatsidis et al. (2008) observed higher anthocyanin content in lettuce 'New Red Fire' and 'Revolution', respectively, when exposed to both UV-B and visible light relative to visible light alone. A positive correlation was also observed between *CHS*, *F3H*, and *DFR* expression and anthocyanin content in lettuce leaves following UV-B exposure (Park et al., 2007).

Other species have also shown a positive association between UV-B exposure and anthocyanin accumulation. *Pinguicula vulgaris* had a 120% increase in anthocyanins

(epidermal cells only) following supplemental UV-B exposure (Mendez et al., 1999). Anthocyanin content in leaf discs of *Rumex patientia* L. and leaves of three near isogenic lines (NILS) of *Oryza sativa* L. (rice) leaves exhibited a significant fluence response to increasing UV-B concentration (Lindoo and Caldwell, 1978; Maekawa et al., 2001). Pea, tomato, and wheat seedlings (Alexieva et al., 2001; Brandt et al., 1995) and *Cotinus coggygria* 'Royal Purple' leaves (Oren-Shamir and Levi-Nissim, 1997a) have also been reported to have increased anthocyanin content following UV-B exposure. Over a five-year period, *Zea mays* inbred lines grown in Chile had higher anthocyanin content than plants grown in Hungary, which was attributed to greater (on average, 28% higher) UV-B exposure in Chile (Pintér et al., 2007).

Not all plant species exhibit a positive relationship between anthocyanin content and UV-B exposure. Beckwith et al. (2004) did not observe any significant increase in anthocyanins in *Pennisetum setaceum* 'Rubrum' following either supplemental UV-A or UV-B exposure (up to 4 h per day for 20 d), but did note that the leaves looked faded by the end of the exposure interval. Anthocyanin content of developing *Liquidambar styraciflua* leaves was unaffected by up to 5 $kJ \cdot m^{-2} \cdot d^{-1}$ of supplemental, biologically-effective UV-B radiation (Dillenburg et al., 1995). *Cichorium intybus* anthocyanin content was unaffected by UV radiation, except at cool temperatures. At 15/10 °C and 20/15 °C, 0% UV transmittance resulted in lower anthocyanin content than 100% UV transmittance (Boo et al., 1997). Moorthy and Kathiresan (1997) noted decreased levels of anthocyanins following UV-B exposure in *Rhizophora apiculata* (mangrove), despite the fact that levels of phenols and other flavonoids increased.

UV-B appears to be necessary, in conjunction with low temperatures, to induce leaf pigmentation in *Cotinus coggygria* 'Royal Purple'. Under low temperature conditions, anthocyanin accumulation will not occur unless plants are also exposed to UV light (Oren-Shamir and Levi-Nissim, 1997b).

Temperature

Anthocyanins have been observed to accumulate in plants in response to low temperatures (Dixon and Paiva, 1995; Tokuhisa et al., 1997). Some mutant *Arabidopsis thaliana* with impaired freezing tolerance [*Sensitivity to freezing3* (*sfr3*), *sfr4, sfr6,* and *sfr7*] also have reduced anthocyanin accumulation following cold acclimation (4 °C, 8 h photoperiod, 220 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PAR*), between 4% and 62% of the anthocyanin content of the wild-type (McKown et al., 1996). As mentioned previously, Chalker-Scott (1999) proposed that the accumulation of anthocyanins during cold stress may provide cold tolerance by functioning as compatibile solutes, delaying freezing and dessication of cells. For example, *Hedera helix* leaves accumulated anthocyanins rapidly during autumn and remained pigmented until spring, which coincided with fluctuations in leaf sugar content (Parker, 1962). However, it appears that while anthocyanin content may increase in response to cool temperatures, it is not necessary for freezing tolerance in *Poncirus trifoliata* (Tignor et al., 1997) or *Arabidopsis thaliana* (Leyva et al., 1995). Therefore, the increase in anthocyanin content may be an acclimation mechanism and they may function as a light screen to help alleviate stress in photosynthetic cells during prolonged exposure to cold temperatures and moderate to high irradiance.

In *Zea mays* it appears that temperature may differentially regulate transcription and biosynthesis of anthocyanins (Christie et al., 1994). In plants exposed to constant temperature (ranging from 5 °C to 20 °C), anthocyanin accumulation was greatest at 15 °C. Following recovery at 25 °C, anthocyanin accumulation more than doubled in plants exposed to 10 °C but did not further increase in plants esposed to 15 °C. Transcript abundance of structural and regulatory anthocyanin biosynthesis genes increased in leaves after exposure to 10 °C, even though anthocyanins did not accumulate until plants were moved to 25 °C, implying that low temperature exposure (10 °C or 15 °C) increased transcription of anthocyanin biosynthesis genes, but enzyme functionality was inhibited at temperatures <15 °C (Christie et al., 1994).

While moderately low temperatures enhance anthocyanin accumulation in a number of species, too low temperatures can completely inhibit anthocyanin biosynthesis. *Zea mays* exposed to 5 °C had suppressed anthocyanin accumulation, even after return to non-chilling temperatures (Christie et al., 1994), and sorghum internodes had reduced anthocyanin content at temperatures less than 8 °C, with maximum accumulation between 16 °C and 20 °C (Shichijo et al., 1993).

High temperature can reduce anthocyanin content in some species. In pears, color loss prior to harvest was greater at higher temperatures (Steyn et al., 2005), primarily due to increased rates of anthocyanin degradation relative to biosynthesis. Grape berry skins accumulated more anthocyanin at 20 °C than 30 °C, which corresponded with increased mRNA levels of both structural and regulatory genes at the higher temperature (Yamane et al., 2006). In field plantings of lettuce, early summer plantings had lower leaf

anthocyanin content than late summer plantings, which was attributed to the higher temperatures experienced by the early summer plantings (Gazula et al., 2007). In a controlled environment, leaf anthocyanin content in lettuce cultivars decreased as temperature increased (from a constant 20 °C to 30/20 °C day/night to constant 30 °C, respectively) (Gazula et al., 2005). Although there was an interaction between cultivar and temperature, this was due to differences in the magnitude of their responses rather than a change in rank order.

Foliar anthocyanin content in *Ipomoea batatas* decreased as the growing temperature increased from 20 to 30 °C (Islam et al., 2005), and anthocyanin content decreased in *Cichorium intybus* as temperature increased (from 15/10 °C day/night to 30/25 °C at 5 °C intervals) (Boo et al., 1997). Leaf anthocyanin content in *Zea mays* exposed to 18 °C was 35-fold higher than at 23 °C (Pietrini and Massacci, 1998). Over a range of day/night temperature combinations, anthocyanin content in *Tagetes erecta* L. (French marigold) leaves decreased as temperature exposure (measured as cumulative degrees per day) increased (Armitage and Carlson, 1981). In *Arabidopsis thaliana* plants overexpressing *PAP1*, leaf coloration was purple at room temperature (normal phenotype) but almost completely green after transfer to high temperature/low light conditions of 30 °C/150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Rowan et al., 2009).

While less common, no change or an increase in anthocyanin content in response to elevated temperature have been reported. Temperature had no significant impact on anthocyanin content or the expression of structural anthocyanin biosynthesis genes in *Capsicum annuum* plants over the range of temperatures evaluated (20/15, 23/18, and

30/25 °C day/night) (Lightbourn et al., 2007). In *Perilla frutescens*, mRNA levels of structural genes were greater at 25 °C relative to 15 °C following a dark pre-treatment (Gong et al., 1997). Anthocyanin content of *Centaurea cyanus* L. cell suspension cultures increased as the culture temperature increased (pre-treatment at 25 °C followed by culture at 5, 15, or 25 °C) (Takahashi et al., 1991). Anthocyanin content per unit fresh weight of *Fragaria ananassa* cv. Shikinari (strawberry) suspension cultures increased as temperature decreased (13-fold higher at 15 °C than at 35 °C), but cell growth increased as temperature increased; therefore, total anthocyanin production ($mg \cdot L^{-1}$) was greatest at moderate temperatures (Zhang et al., 1997). And in coleus, a cultivar-specific response to temperature was observed. In two of the three red-leaved cultivars ('Big Red Judy' and 'Twist and Twirl' red), anthocyanin content was greatest at 12 °C (relative to 18, 24, or 30 °C), but in 'Royal Glissade', anthocyanin content was lowest at 12 °C (Boldt et al., 2011a).

Leaf maturity may impact responsiveness to changes in temperature. Both young and mature leaves of *Cotinus coggygria* 'Royal Purple' had decreased leaf pigmentation when plants were transferred to higher temperatures (17/9 °C day/night for 21 d, then moved to 29/21 °C day/night), but only young leaves accumulated anthocyanin when plants were transferred to lower temperatures (29/21 °C day/night for 21 d, then moved to 17/9 °C day/night) (Oren-Shamir and Levi-Nissim, 1997b). Plant developmental stage may also affect anthocyanin accumulation in response to temperature. No *Arabidopsis thaliana* ecotypes accumulated anthocyanins in response to chilling (14 °C) or cold (6 °C) temperatures during the vegetative stage, but during the reproductive stage of growth,

some ecotypes had elevated anthocyanin content at 6 °C or 14 °C relative to plants grown at 22 °C (Hasdai et al., 2006).

Nutrients

Phosphorus deficiency is most commonly associated with anthocyanin accumulation, but nitrogen and potassium deficiencies have also been noted to induce anthocyanin formation (Hodges and Nozzolillo, 1996; Lawanson et al., 1972; Yuan et al., 2009). Foliar applications of boron (0.8 $mg \cdot L^{-1}$) or calcium (300 $mg \cdot L^{-1}$) accelerated pigmentation in transitional bracts of *Euphorbia pulcherrima*, but did not significantly affect final anthocyanin concentration (Arreola et al., 2008). *Spirodela polyrhiza* L. Schleid. (duckweed) grown in Hoagland's nutrient solution accumulated more anthocyanins as the solutions became more dilute (44% increase in the 1:80 dilution relative to the 1:5 dilution) (Kumar and Sharma, 1999).

Phosphorus deficiency symptoms appear first in older leaves because P is a mobile element, and the symptoms progress upwards in a plant as the deficiency worsens (Havlin et al., 2005). In red cabbage, a species with a constitutive baseline level of anthocyanin pigmentation, no differences in anthocyanin content were observed in field-grown plants in response to various P application rates (Piccaglia et al., 2002), but Yuan et al. (2009) observed a 1.8-fold increase in anthocyanin content in red cabbage seedlings grown hydroponically without P relative to seedlings grown in a complete Hoagland's solution. Additionally, anthocyanin content in *Solenostemon scutellarioides* (coleus),

another species with constitutive anthocyanin pigmentation, was not correlated with leaf P concentration (Henry et al., 2012), or P application rate (Boldt, unpublished data).

In plants with very little anthocyanin pigmentation under normal growing conditions, a marked increase in anthocyanin content has been observed as a result of reduced P application. Anthocyanin concentration increased almost 30-fold in *Arabidopsis thaliana* seedlings treated with low P (6 $mmol \cdot m^{-3}$) relative to those supplied with high P (1 $mol \cdot m^{-3}$) (Trull et al., 1997), and P deficiency in tomato plants increased anthocyanin content by as much as 1333% (Ulrychová and Sosnová, 1970).

Anthocyanin accumulation in response to P deficiencies has been observed in a diverse array of species: *Arabidopsis thaliana* (Misson et al., 2005; Trull et al., 1997; Zakhleniuk et al., 2001); three *Flaveria* species (Halsted and Lynch, 1996); limpograss (Shaikh et al., 2008); cabbage, cauliflower, and canola (Hodges and Nozzolillo, 1996); grape cell suspension cultures (Dedaldechamp et al., 1995); and *Zea mays* (Lawanson et al., 1972). Increased anthocyanin content has been observed in conjunction with decreased shoot P in *Zea mays* (Cobbina and Miller, 1987) and sunflower (r = -0.64) (Gunes and Inal, 2009), and Atkinson (1973) noted an increase in leucoanthocyanin content in response to P deficiency in 21 dicot and 24 monocot species.

Drought/Salinity

Anthocyanin content in maize roots decreased following the application of a 1% or 2% solution of KCl or NaCl when expressed on a per root basis but increased in the 2% KCl and NaCl treatments on a per dry weight basis (Kaliamoorthy and Rao, 1994).

Salinity also had differential effects on anthocyanin content in *Bellis perennis* L. Plants supplemented with 15 or 25 mM NaCl for 40 d had increased anthocyanin content, while plants supplemented with 50 or 75 mM NaCl had decreased anthocyanin content (Khavari-Nejad et al., 2008).

Carbohydrates

Anthocyanins are glycosylated anthocyanidins, with one or more sugar moieties attached, and the application of exogenous sugar may increase anthocyanin content. In non-chlorophyllous *Zea mays*, the application of supplemental sucrose increased transcription of anthocyanin biosynthesis genes (*CHS*, *CHI*, *F3H*, *DFR*, and *ANS*) (Kim et al., 2006). The addition of 0.05 M sucrose increased anthocyanin content in grape leaf discs, especially in conjunction with ABA (20 µM) or ethylene (60 µM) (Pirie and Mullins, 1976). In *Arabidopsis thaliana*, anthocyanin content was similar in plants supplied with 0, 4, or 20 mM supplemental carbon but was 2.3-fold higher in plants supplemented with 100 mM compared to those supplied with 0 mM (Stevenson and Harrington, 2009). However, the type of supplemental sugar provided differentially affected anthocyanin content. Anthocyanin accumulation, relative to a combined osmotic control, was higher in *Arabidopsis thaliana* supplemented with galactose or mannose, lower in plants supplemented with arabinose, and similar in plants supplemented with fructose, fucose, glucose, maltose, rhamnose, sucrose, or xylose (Stevenson and Harrington, 2009).

Plant Growth Regulators

Cytokinins can increase anthocyanin content in *Arabidopsis thaliana*. Seedlings grown on media containing kinetin or zeatin had increased anthocyanin content (Chen et al., 2006), and benzyladenine (BA) increased anthocyanin content in hypocotyls, stems, and petioles and resulted in elevated mRNA levels of *PAL1*, *CHS*, *CHI*, and *DFR* (Deikman and Hammer, 1995). In *Zea mays*, however, BA was inhibitory (Kim et al., 2006). Auxin (up to 114 µM IAA) did not stimulate anthocyanin accumulation in *A. thaliana* (Deikman and Hammer, 1995).

Ethylene was ineffective at stimulating anthocyanins in *A.thaliana* (1 $\mu L \cdot L^{-1}$) and grape leaf discs (60 µM) (Deikman and Hammer, 1995; Pirie and Mullins, 1976). Abscisic acid (ABA) was effective, however, in inducing anthocyanin accumulation in grape leaf discs (20 µM) and *Z. mays* (0.01 to 1.0 µM) (Kim et al., 2006; Pirie and Mullins, 1976).

Gibberellic acid (GA) was inhibitory to anthocyanin accumulation in *Zea mays* and *Daucus carota* cell suspension cultures (Ilan and Dougall, 1992; Kim et al., 2006), but this was reversible in *D. carota* by the application of paclobutrazol, a GA antagonist. Paclobutrazol, uniconazole, ancymidol, and chlormequat chloride (all GA inhibitors) increased anthocyanin content in *D. carota* cell suspension cultures (Ilan and Dougall, 1992).

Future Research

Foliage coloration in ornamentals has become more popular with consumers in recent years due, in part, to an increased color and texture palate, minimal maintenance requirements relative to flowering plants (no need to remove dead flowers), and season-long coloration. However, no matter how well a plant performs in the landscape, it must first be visually appealing at retail markets and purchased by consumers. Determining which environmental factors influence anthocyanin accumulation, which ones have the greatest impact upon foliage coloration, and how quickly anthocyanin content can be altered can help in the development of grower recommendations so that plants can have optimal foliage coloration when shipped to retail.

Second, some of the health benefits of fruit and vegetable consumption have been attributed to the presence of flavonoids (including anthocyanins) (Stintzing and Carle, 2004). Through breeding efforts and a better understanding of how the environment influences plant phenotype, the anthocyanin content of many fruits has been increased. Increasing the anthocyanin content of vegetables (and herbs) can provide the same health benefits, and results foliar anthocyanin research can be extended to the production of red leafy vegetables and herbs with both increased aesthetic value and health properties.

Lastly, further research on differences in photosynthetic capacity and photosynthetic efficiency between red and green-leaved plants can potentially offer avenues for breeders to breed plants better able to withstand environmental stresses. If red leaves have higher rates of photosynthesis and less photosystem damage following

exposure to stress, red phenotypes may be able to produce more dry matter than green phenotypes when grown in marginal environments.

Gould (2004) stated in a review article that "anthocyanins offer multifaceted, versatile, and effective protection to plants under stress. They are the Swiss army knife of the plant kingdom." Continued research may offer further insights into the physiological basis for where and why anthocyanins accumulate in leaves and how they may protect leaves during stress.

Table 1.1. Major types of anthocyanidins extracted from leaves, stems, bracts, or cell suspension cultures of anthocyanic species.

Species	Anthocyanidin				Reference
	Cyanidin	Delphinidin	Malvidin	Peonidin	
Acer platanoides	x				Merzlyak et al., 2008
Apium graveolens	x				Timberlake and Bridle, 1982
Ambrosia chamissonis	x				Page and Towers, 2002
Arabidopsis thaliana	x				Bloor and Abrahams, 2002; Tohge et al., 2005
Brassica juncea				x	Timberlake and Bridle, 1982
Brassica oleracea	x				Timberlake and Bridle, 1982; Yuan et al., 2009
Capsicum annuum		x			Stommel et al., 2009
Centaurea cyanus	x				Takahashi et al., 1991
Cichorium intybus	x				Boo et al., 1997
Coffea arabica		x			Domingues et al., 2012
Coleus blumei	x				Lawrence et al., 1939; Robinson and Robinson, 1931
Cornus alba	x				Merzlyak et al., 2008
Corylus avellana	x				Merzlyak et al., 2008
Cotoneaster alaunica	x				Merzlyak et al., 2008
Euphorbia pulcherrima	x				Asen, 1958
Foeniculum vulgare	x				Timberlake and Bridle, 1982
Lactuca sativa	x				Park et al., 2008
Medicago sativa	x				Ray et al., 2003
Ocimum basilicum	x				Phippen and Simon, 1998
Parthenocissus quinquefolia	x				Merzlyak et al., 2008
Perilla frutescens	x				Nishimura et al., 2009; Saito and Yamazaki, 2002
Quintinia serrata	x				Gould et al., 2002
Rheum rhaponticum	x				Timberlake and Bridle, 1982
Syzygium leuhmannii			x		Woodall and Stewart, 1998
Syzygium wilsonii			x		Woodall and Stewart, 1998
Vitis vinifera	x				Dedaldechamp et al., 1995
46 polygonaceous species	x				Yoshitama et al., 1987
23 grass species	x				Fossen et al., 2002

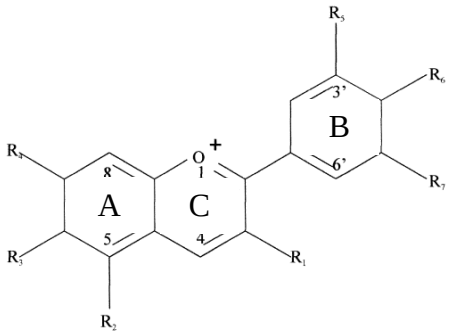

Fig. 1.1. Basic anthocyanidin structure, composed of two phenolic rings in a C_6-C_3-C_6 arrangement. At each R position, H, OH, or CH_3 may be attached depending upon the anthocyanidin. Glycosylation is common at C_3 (R_1) (adapted from Delgado-Vargas et al., 2000).

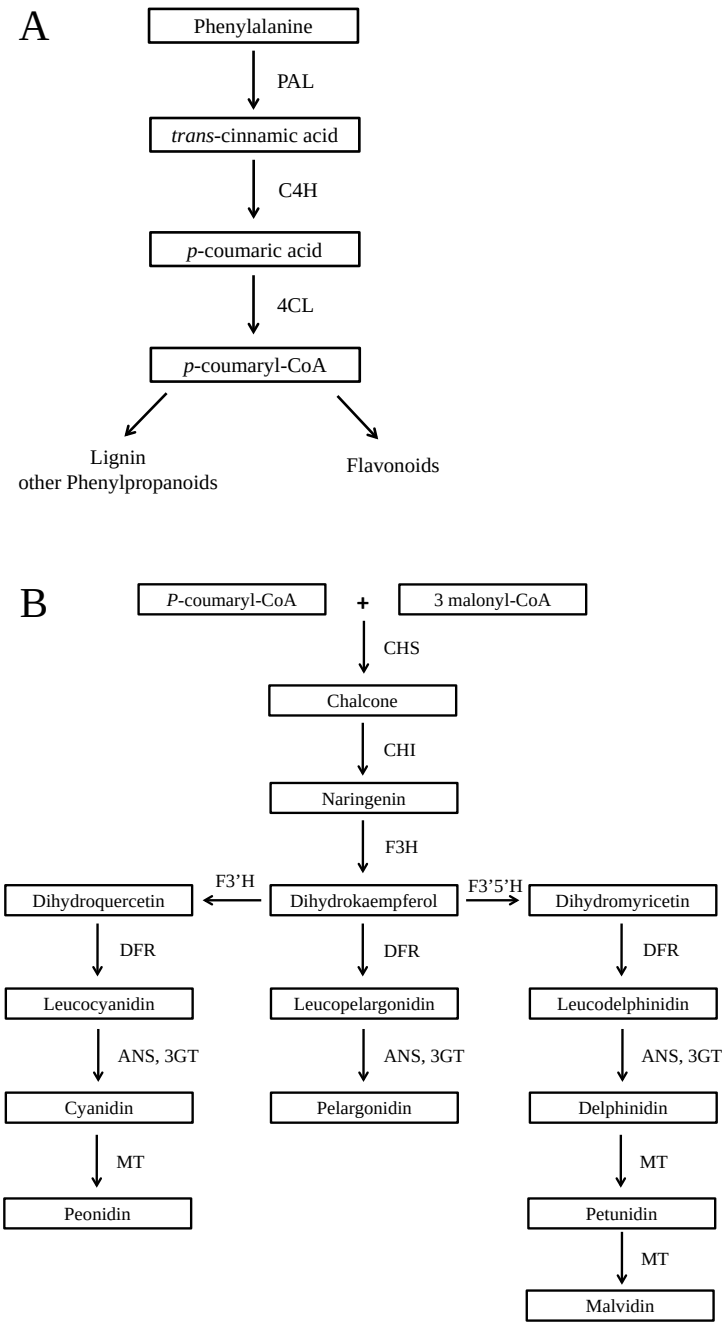

Fig. 1.2. Phenylpropanoid (A) and flavonoid (B) biosynthetic pathways. PAL: phenylalanine ammonia lyase, C4H: cinnamate-4-hydroxylase, 4CL: 4-coumaryl:CoA ligase, CHS: chalcone synthase, CHI: chalcone isomerase, F3H: flavanone-3-hydroxylase, F3'H: flavanone-3'-hydroxylase, F3'5'H: flavanone-3',5'-hydroxylase, DFR: dihydroflavonol-4-reductase, ANS: anthocyanidin synthase, 3GT: glucosyl transferase, MT: methyl transferase (adapted from Delgado-Vargas et al., 2000).

Chapter 2

Anthocyanin and Chlorophyll Show Seasonal Variation in Grasses

Many ornamental grasses have red foliage, which makes them attractive in the landscape. We analyzed the seasonal variation of anthocyanin and chlorophyll in grasses that vary in leaf coloration. Leaves of *Imperata cylindrica* (L.) P. Beauv. 'Red Baron'; *Panicum virgatum* L. 'Northwind', 'Prairie Fire', 'Red Sunset', 'RR1' Ruby Ribbons™, and 'Shenandoah'; *Pennisetum advena* Wipff & Veldkamp; *Pennisetum purpureum* Schumach. 'Prince'; and *Schizachyrium scoparium* (Michx.) Nash 'MinnBlueA' Blue Heaven™ and a variegated form, were sampled every two weeks (from early July to late September) over three years (2009-11) for anthocyanin and chlorophyll content. In the red-leaved taxa evaluated in this study, the intensified leaf coloration observed as the growing season progressed resulted from an increase in anthocyanin content and a decrease in total chlorophyll content. In green-leaved *P. virgatum* 'Northwind', anthocyanin content remained constant and chlorophyll content increased throughout the sampling period. Average daily temperature (ADT, °C) was negatively correlated with anthocyanin concentration in all but one taxa (r = 0.01 to -0.67) and total growing degree days (GDD) was positively correlated with anthocyanin concentration (r = 0.07 to 0.88). Likewise, anthocyanin concentration was negatively correlated with average daily light integral (DLI; r = -0.04 to -0.79) and positively correlated with total DLI (r = 0.08 to 0.83). Chlorophyll content was negatively correlated with ADT in *P. virgatum*

'Northwind' and variegated *S. scoparium* (r = -0.26 and -0.08, respectively) but positively correlated with ADT in the other taxa (r = 0.41 to 0.88). In all taxa (except *P. virgatum* 'Northwind'), chlorophyll content was positively correlated with mean DLI (r = 0.04 to 0.75) and negatively correlated with total DLI (r = -0.29 to -0.90). We observed that the annual and non-native grass species had minimal seasonal increases in anthocyanin content or decreases in total chlorophyll content relative to the two native grass species (*P. virgatum* and *S. scoparium*). The increase in anthocyanin detected in late summer and autumn might be an adaptive mechanism in these native, perennial grasses.

Introduction

Leaf pigmentation may be uniform or variable across a leaf surface, as seen on variegated foliage (Chalker-Scott, 1999; Manetas, 2006). It can also vary during the growing season. Leaf coloration often appears in juvenile leaves of many tropical species, in mature leaves following exposure to stress, and in deciduous tree and shrub species in autumn (Close and Beadle, 2003). Permanent leaf coloration has been observed in species ranging across a diverse array of latitudes, altitudes, and habitats (Gould, 2004). In most plants, red and burgundy foliage color is due to the presence of anthocyanins (Tanaka et al., 2008).

Anthocyanins belong to a group of secondary compounds known as flavonoids (Chalker-Scott, 1999). They are ubiquitous in plants, occurring in flowers, fruits, seeds, leaves, stems, and roots. While they attract pollinators to receptive flowers and seed-

dispersal animals to mature, ripe fruit (Steyn et al., 2002), the basis for anthocyanin accumulation in leaves is still not entirely understood. However, there is evidence that they may serve different functions in leaves of different species. Major functions attributed to leaf anthocyanins include deterring herbivores (Lev-Yadun et al., 2004), protecting against photodamage (due to high irradiance, low temperatures, or nutrient deficiencies) (Gould, 2004; Manetas, 2006), and scavenging free radicals (Neill and Gould, 2003). In all cases, their occurrence in leaves may help protect against potential photosynthetic losses due to leaf loss by herbivory or reduced rates of photosynthesis due to photoinhibition.

Ornamental grasses are grown for their range of growth habits, flower color, inflorescence shape, leaf size and shape, and leaf color. In some genera (e.g. *Pennisetum*), foliar coloration is permanent throughout the growing season but may vary in intensity depending upon environmental conditions. Beckwith et al. (2004) observed that light quality and light intensity affected anthocyanin accumulation in *Pennisetum setaceum* 'Rubrum' and 'Red Riding Hood'. In other genera, including *Panicum* and *Schizachyrium*, leaf coloration is transient to semi-permanent. Plants emerge in the spring with green leaves and slowly turn red during late summer and early autumn. Individual leaves transition from green to red, and the percentage of leaves in the plant canopy with red pigmentation also increases. In this study, we evaluated the seasonal variation in anthocyanin and chlorophyll content in five grass species across three growing seasons to answer the following research questions: Does the increase in red leaf coloration occur as a result of increased anthocyanin content or decreased chlorophyll

content, resulting in an unmasking of anthocyanins already present in leaf tissue? Do species differ in seasonal anthocyanin and chlorophyll content? Are seasonal pigmentation patterns consistent across multiple years?

Materials and Methods

Leaf samples from five ornamental grass species (10 taxa in total) were collected every two weeks during the growing season (weeks 28 to 40, approximately the second week in July to the fourth week in September) for three years (2009-11). Taxa used in this study were *Imperata cylindrica* 'Red Baron' (Japanese bloodgrass or cogon grass); *Panicum virgatum* 'Northwind', 'Prairie Fire', 'Shenandoah', 'Red Sunset', and 'RR1' Ruby Ribbons™ (switchgrass); *Pennisetum advena* (purple fountaingrass) (2009 only); *Pennisetum purpureum* 'Prince' (elephant grass) (2009 only); and *Schizachyrium scoparium* 'MinnBlueA' Blue Heaven™ (little bluestem) and a dwarf, green and white variegated form of *S. scoparium*. Plants were at the Minnesota Landscape Arboretum (Chaska, MN) except for *I. cylindrica* 'Red Baron', which was located at Noerenberg Gardens (Three Rivers Park District, Wayzata, MN), a distance of less than 8 miles from the other taxa. *P. advena* and *P. purpureum* had anthocyanic leaves at all harvests, and *P. virgatum* 'Northwind' had non-anthocyanic (acyanic) leaves at all harvests. The remaining taxa had a mix of acyanic and anthocyanic leaves for the first few harvests, then solely anthocyanic leaves by the end of the season.

Nine leaves exposed to full sunlight were randomly sampled from each taxa at each harvest. Leaf samples were collected approximately 25% from the leaf tip, where the diameter of the leaf blade was wide enough for sample collection but not affected by shading from the plant canopy. Two leaf discs (each 0.3 cm^2), one for anthocyanin and one for chlorophyll content, were collected from each leaf using a hole punch, placed in foil packets, and stored on dry ice until they could be stored at -80 °C until pigment extraction. Each leaf disc was placed in a 1.7 mL microcentrifuge tube containing either 1 mL of 99:1 (v:v) methanol:HCl (for anthocyanin extraction) or 95% ethanol (for chlorophyll extraction) and two tungsten carbide beads. The leaf tissue was homogenized in a Mixer Mill (MM 300; Qiagen, Valencia, CA), then placed in the dark at 4 °C for 18 h. Samples were centrifuged (model 5714R; Eppendorf, Hamburg, Germany) at 4 °C for 10 min at 10,000 *g*. A 300 µL aliquot from each microcentrifuge tube was pipeted into a 96-well plate and absorbance was measured using a plate reader with monochromater optics (SpectraMax 190; Molecular Devices, Sunnyvale, CA). Absorbance for anthocyanin was measured at 530 nm for all taxa, and chlorophyll was measured at 649 and 665 nm. Absorbance values were normalized to a 1 cm pathlength and 1 cm^2 leaf area. Anthocyanin content was calculated using an extinction coefficient (ε) of 34,300 for cyanidin-3-glucoside (Siegelman and Hendricks, 1958), and chlorophyll was calculated using equations published by Wintermans and De Mots (1965).

Temperature and precipitation data were collected from the National Weather Service weather station in Chanhassen, MN (3 miles from the Minnesota Landscape Arboretum), and sunlight data (daily light integral, DLI) were collected from the

University of Minnesota, St. Paul campus weather station (25 miles away) (Fig. 2.1). Growing degree days (GDD, °C) were calculated as $[(T_{max}-T_{min})/2]-T_{min}$, with 10 °C as T_{min} (the base temperature) and no upper threshold (T_{max}) since all taxa are warm-season (C_4) grasses (Sanderson and Wolf, 1995). Average daily temperature (ADT, °C) and mean DLI ($mol \cdot m^{-2} \cdot d^{-1}$) were calculated for the 2-week period preceding each harvest. Cumulative GDD, DLI, and precipitation were calculated starting May 1 of each year, corresponding to the approximate date of leaf emergence in perennial grasses in our study.

In all three years, no insect, fungal, or disease incidences which may have affected plant growth or pigment accumulation were observed. Plants were not provided supplemental irrigation or fertilized during the experiment

The experimental layout was a split plot, with year as the main plot, cultivar as the sub-plot, and repeated measures (harvest week) on the sub-plot. Data were analyzed using PROC MIXED in SAS 9.3 (Cary, NC) and mean separation was conducted for significant sources of variation using Tukey's HSD at $\alpha=0.05$.

Results

Anthocyanin content

Taxa differed in anthocyanin content across harvest week and year (Table 2.1). Anthocyanin content ($\mu g \cdot cm^{-2}$) in all four red *P. virgatum* cultivars increased as harvest week increased in all three years (Fig. 2.2B-E). There was less variability between years at early harvest intervals (weeks 28-34), with increased year to year variability at later

harvests (weeks 36-40). In general, during weeks 38 and 40, anthocyanin content in the red-leaved *P. virgatum* cultivars was higher in 2010 than in 2009 or 2011. *P. virgatum* 'Northwind' leaves contained some anthocyanin (between 7.0 and 12.1 $\mu g \cdot cm^{-2}$), although they remained green throughout the sampling period every year (Table 2.2).

Anthocyanin content in *S. scoparium* Blue Heaven™, like *P. virgatum*, increased as harvest week increased at the early harvest intervals (weeks 28-34) and showed a similar pattern across years (Fig. 2.2G). At the later harvest weeks, anthocyanin content was more variable. The variegated little bluestem had lower anthocyanin content than Blue Heaven™ and was less variable across years and harvest weeks (Fig. 2.2H). *I. cylindrica* 'Red Baron' anthocyanin accumulation was more constant across harvest dates than *P. virgatum* (Fig. 2.2F). In all three years, little variation was observed from week 30 to week 38. Data were not collected for week 40 in 2011 due to the removal of plant material from the harvest site between weeks 38 and 40.

Both *P. advena* and *P. purpureum* 'Prince', annuals in most climates, were grown in 2009 only. With both, anthocyanin content increased from week 28 to week 32, decreased from week 32 to week 36, then increased slightly from week 38 to week 40 (Fig. 2.2I-J).

Relative to the historical 30-year average, 2009 had lower than average summer temperatures (up to week 36) and higher than average autumn temperatures (September), while 2010 and 2011 generally had higher than average summer temperatures (July and August) and lower than average temperatures in September (Fig. 2.1A-C). Between weeks 28 and 36, the ADT was 2.1 °C below average in 2009 and 1.0 °C above average

in both 2010 and 2011. In weeks 38 and 40, ADT was 2.3 °C above average in 2009 and 1.0 °C below average in 2010 and 2011, respectively.

Average DLI also varied across year and harvest week (Fig. 2.1D). For example, 2011 had the highest DLI of all three years in the period prior to the week 28 harvest but the lowest DLI of all years in the following two harvest intervals (weeks 30 and 32). Average DLI in all three years decreased as the season progressed from summer to autumn, ranging from 39.2-46.4 $mol \cdot m^{-2} \cdot d^{-1}$ in week 28 to 20.1-22.5 $mol \cdot m^{-2} \cdot d^{-1}$ in week 40.

To account for yearly variations in environmental conditions, correlations between pigment concentration and temperature and irradiance were examined (Table 2.3). ADT was negatively correlated with anthocyanin concentration in all but one taxa (r = 0.01 to -0.67) and total GDD (accumulated from May 1) was positively correlated with anthocyanin concentration (r = 0.07 to 0.88). Likewise, anthocyanin concentration was negatively correlated with average DLI (r = -0.04 to -0.79) and positively correlated with total DLI (r = 0.08 to 0.83).

Chlorophyll content

Chlorophyll content varied among taxa across harvest week and year (Table 2.1). In green-leaved *P. virgatum* 'Northwind', chlorophyll content generally increased throughout the sampling period. For example, in 2009, it increased from 46.2 $\mu g \cdot cm^{-2}$ in week 28 to 64.1 $\mu g \cdot cm^{-2}$ in week 40 (Fig. 2.2A). Chlorophyll content decreased in the four anthocyanic *P. virgatum* cultivars from summer to autumn (Fig. 2.2B-E).

Chlorophyll content in *I. cylindrica* was very low relative to other taxa in the study (Fig. 2.2F), except for the variegated *S. scoparium* (Fig. 2.2H). Maximum chlorophyll content in *I. cylindrica* was 15.2 $\mu g \cdot cm^{-2}$ in week 28 (2009) but typically ranged between 4 and 10 $\mu g \cdot cm^{-2}$, while chlorophyll content in anthocyanic *P. virgatum* cultivars, *Pennisetum*, and *S. scoparium* Blue Heaven™ typically ranged between 15 and 50 $\mu g \cdot cm^{-2}$ (maximum chlorophyll content for individual taxa ranged between 31.4 and 54.8 $\mu g \cdot cm^{-2}$; Table 2.4).

Chlorophyll content in *S. scoparium* Blue Heaven™ decreased from summer to autumn, whereas in the variegated little bluestem, chlorophyll content remained relatively constant from weeks 28 to 40 (Fig. 2.2G and H). The variegated little bluestem had lower chlorophyll content than Blue Heaven™, which was not unexpected, given its variegation pattern (white and green bicolor, with pink coloration developing in late summer and early autumn) and reduced growth rate (smaller leaves, compact growth habit, reduced overwintering survivability). The two annual *Pennisetum* species had constant chlorophyll content from weeks 28-34, then exhibited a late-season decline (Fig. 2.2I and J).

As with anthocyanin, correlations between chlorophyll content and environmental conditions were examined for yearly variations in temperature and irradiance during the sampling period (Table 2.3). Chlorophyll content was negatively correlated with ADT in *P. virgatum* 'Northwind' and variegated *S. scoparium* (r = -0.26 and -0.08, respectively), but positively correlated in other taxa (r = 0.41 to 0.88). In all taxa except *P. virgatum* 'Northwind', total GDD was negatively correlated with chlorophyll content (r = -0.29 to

-0.90). Similar to ADT and total GDD, chlorophyll content was positively correlated with average DLI and negatively correlated with total DLI in all cultivars except *P. virgatum* 'Northwind' (Table 2.3).

Discussion

In the red-leaved taxa evaluated in this study, the intensified leaf coloration we observed as the growing season progressed resulted from an increase in anthocyanin content and a decrease in total chlorophyll content. This response was most obvious in the anthocyanic *P. virgatum* cultivars and *S. scoparium* Blue Heaven™. *I. cylindrica* and variegated *S. scoparium* showed only minor fluctuations in anthocyanin and chlorophyll content over the 12-week sampling period.

Low temperature and high irradiance can promote anthocyanin accumulation over periods as short as a few days to a few weeks (Beckwith et al., 2004; Gazula et al., 2005; Islam et al., 2005; Kleinhenz et al., 2003). When we examined the relationship between anthocyanin content and environmental conditions in the 2-week period preceding each harvest, a negative correlation was observed between ADT and anthocyanin content in all *P. virgatum* cultivars, both *S. scoparium* taxa, and *I. cylindrica* (r = -0.35 to -0.68), but not in the *Pennisetum* species. The increase in anthocyanin accumulation in most of our grass taxa following exposure to lower temperatures (i.e., lower ADT) is consistent with results observed in *Zea mays* (Christie et al., 1994; Pietrini and Massaci, 1998), *Lactuca sativa* (Gazula et al., 2005, 2007), and *Ipomoea batatas* (Islam et al., 2005). Total GDD,

however, had the opposite effect (a positive correlation, r = 0.07 to 0.88) on anthocyanin content since GDD accumulates during the growing season and is lowest at the start of a growing season and greatest at the end.

In general, anthocyanin content increased across the 12-week sampling period, but average DLI declined from summer to autumn. As a result, average DLI was negatively correlated with anthocyanin content in all taxa (r = -0.04 to -0.79). This is contrary to what has been reported for many species; anthocyanin content typically increases in response to increasing irradiance (Beckwith et al., 2004; Hughes et al., 2005; Islam et al., 2005; Kleinhenz et al., 2003; Lightbourn et al., 2007; Richards et al., 2004). For example, in our own research, anthocyanin content in *P. virgatum* Ruby Ribbons™ increased as light intensity increased (irradiance ranged from 75 to 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (Boldt et al., 2011a). Total DLI, however, was positively correlated with anthocyanin content (r = 0.08 to 0.83) as a result of the increase in total accumulated irradiance as the growing season progressed.

Over the course of a growing season, total accumulated GDD and DLI will increase, while ADT and average DLI will decrease, due to the seasonal shift from summer to autumn. The decrease in average DLI was the combined result of decreased daylength and light intensity. Daylength decreased from approximately 15:30 h in week 28 to approximately 11:45 h in week 40 (USNO, 2012), and irradiance was less intense in later weeks as the sun angle decreased from summer to autumn. When average light intensity was calculated, accounting for daylength, there was still a negative relationship

between average instantaneous light intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$) per day and anthocyanin content (data not shown).

A negative correlation between anthocyanin content and ADT agrees with previous research in other species, but a negative correlation with average DLI contradicts previous research, including one of our own studies with *P. virgatum* Ruby Ribbons™ (Boldt et al., 2011a). One possibility is that temperature had a greater impact on regulating anthocyanin biosynthesis than irradiance in these grass taxa. In most studies, the effects of irradiance and temperature on anthocyanin content are examined singly, independent of one another (Beckwith et al., 2004; Islam et al., 2005; Richards et al., 2004). In *Cichorium intybus* L., the combined impacts of temperature and light were investigated. An increase in anthocyanin content at higher irradiances was more pronounced at lower temperatures, with no change in anthocyanin content between 50 and 340 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 30/25 °C day/night temperatures (Boo et al., 1997). Maximum anthocyanin content occurred at low temperature and high irradiance in *Cichorium intybus* but at low ADT and low DLI in the grass taxa in this study. Because low ADT and low DLI are seasonally linked, it is unknown how irradiance, in the absence of temperature fluctuations, would have influenced anthocyanin accumulation in field-grown plants.

In field experiments, temperature and irradiance fluctuate constantly (hourly, daily, and seasonally), and plants are also subject to additional environmental cues that can regulate intrinsic signals (e.g. photoperiodic flowering or the initiation of autumn coloration in temperate trees and shrubs). Therefore, it is possible that, in the grass taxa

we studied, external cues may trigger internal signals involved in regulating seasonal anthocyanin accumulation and chlorophyll content, independent of the "stress accumulation" of anthocyanins in response to low temperature and high irradiance.

Anthocyanins may accumulate in juvenile, mature, and senescing leaves (Close and Beadle, 2003). The appearance of red leaves during leaf expansion and senescence is controlled by developmental cues (Manetas, 2006), which allow the leaves to be competent to accumulate anthocyanins. Additional environmental cues control the intensity of pigmentation (Manetas, 2006). Anthocyanin accumulation in young, developing leaves is more common in tropical climates and accumulation in autumnal leaves is more common in temperate climates (Dominy et al., 2002; Lee and Collins, 2001; Whedale, 1916). Lee and Collins (2001) examined 399 tropical plant species (collected from Florida, Costa Rica, and Panama) and observed that 45% of the taxa produced anthocyanins during leaf expansion but only 13.5% produced anthocyanins during leaf senescence. In contrast, 70% of woody taxa surveyed in the Harvard Forest (Massachusetts, USA), produced anthocyanins during leaf senescence (Lee et al., 2003).

All of the grass taxa we evaluated are warm-season, C_4 grasses (Buchmann et al., 1996; Christin et al., 2009; Hattersley and Watson, 1976; Sinha and Kellogg, 1996). *P. virgatum* and *S. scoparium* are native to the United States (upper Midwest; USDA, 2012), whereas *I. cylindrica* is native to southeast Asia, and both *Pennisetum* species are native to Africa (USDA, 2012).

Minnesota has a relatively short growing season, with an average of 156 d between the last spring freeze (0 °C; median date = 1 May) and first freeze in autumn

(median date = 2 Oct.) at our research sites (Chaska, MN; MRCC, 2012). Within this period, perennial grasses must emerge, flower and set seed (in most species), and store enough carbohydrates and nutrients to sustain initial growth the following year. In Minnesota, *I. cylindrica*, *P. virgatum*, and *S. scoparium*, are perennials (*I. cylindrica*, however, is a weak perennial and rarely flowers) and *P. advena* and *P. purpureum* are annuals. We observed that the annual and non-native grass species had minimal seasonal increases in anthocyanin content or decreases in total chlorophyll content relative to the two native grass species. The increase in anthocyanin detected in late summer and autumn might be an adaptive mechanism in these native, perennial grasses.

None of the species accumulated anthocyanins in developing leaves, as is commonly observed in tropical species. Instead, it appeared as if *P. virgatum* and *S. scoparium* exhibited pigmentation patterns typically seen in autumn foliage coloration. In many temperate, deciduous tree species, *de novo* synthesis of anthocyanins occurs in autumn in conjunction with the dismantling of chloroplasts and recovery of nutrients, especially nitrogen, before leaf senescence occurs (Archetti, 2009). The occurrence of anthocyanins in autumn is believed to help provide photoprotection, as cool temperatures and sunny days can cause extra stress to the photosystems (Hoch et al., 2001). Lee et al. (2003) noted that increases in anthocyanin accumulation in deciduous species coincided with a decline in chlorophyll to less than 20 $\mu g \cdot cm^{-2}$.

When comparing deciduous woody species, those native to the northern USA and Canada had a greater incidence of anthocyanin production than species native to Europe (Hoch et al., 2001). This corresponds to our observation of the two U.S. native species,

P. virgatum and *S. scoparium*, accumulating more anthocyanins than the non-native species. While *P. virgatum* and *S. scoparium* are not deciduous like trees are, they do senesce and their leaves begin to turn brown following the first frost or subzero °C night. This makes the dismantling of chloroplasts and the recovery and translocation of nutrients from leaves in autumn an important step for overwintering survivability. Although anthocyanin accumulation in grasses begins much earlier in the growing season (July) than deciduous tree species (typically September), the increase in anthocyanin content did coincide with a concurrent decline in chlorophyll content. Therefore, because they have a perennial growth habit and have adapted to survive in a northern continental climate, it is plausible that *P. virgatum* and *S. scoparium* have developed a similar pattern of autumnal coloration and the accumulation of anthocyanins serves a photoprotective function in grasses, similar to other deciduous plants.

Table 2.1. Analysis of variance for anthocyanin and total chlorophyll content of 10 grass taxa sampled every 2 weeks for 3 years. The experimental layout was a split-plot, with year as the main plot, taxa as the sub-plot, and repeated measures (harvest week) on the sub-plot.

Factor	df	Anthocyanin		Total chlorophyll	
		F value	P value[z]	F value	P value
Year	2	0.8	0.47	0.7	0.53
Taxa	9	283.3	<0.0001	1099.2	<0.0001
Year x taxa	14	5.6	<0.0001	28.7	<0.0001
Week	6	349.2	<0.0001	297.9	<0.0001
Week x year	12	52.4	<0.0001	22.3	<0.0001
Week x taxa	54	39.2	<0.0001	82.1	<0.0001
Week x taxa x year	83	12.5	<0.0001	20.1	<0.0001

[z] Significant if $P \leq 0.05$.

Table 2.2. Foliar anthocyanin content ($\mu g \cdot cm^{-2}$) for 10 grass taxa varying in foliar coloration. Leaf samples were harvested every 2 weeks (weeks 28-40) for three years (2009-11) to quantify seasonal variation in leaf pigmentation. For each taxa, means ($\pm$ SE) in each column followed by similar uppercase letters and means in each row followed by similar lowercase letters are not significantly different at $P<0.05$ (mean separation performed using Tukey's HSD).

Taxa	Year	Week						
		28	30	32	34	36	38	40
I. cylindrica	2009	11.1 ± 3.3 Bb	21.8 ± 3.0 Aa	21.4 ± 2.1 Aa	21.0 ± 2.0 Aa	13.7 ± 0.8 Ab	20.3 ± 1.7 Aa	11.6 ± 1.0 Bb
'Red Baron'	2010	18.1 ± 1.3 Aa	10.7 ± 0.9 Bb	13.4 ± 1.5 Bb	14.2 ± 1.9 Bb	11.3 ± 0.6 Ab	19.7 ± 1.1 Aa	20.9 ± 1.2 Aa
	2011	18.8 ± 1.9 Aa	13.8 ± 1.4 Bbc	10.2 ± 0.9 Bc	14.7 ± 1.5 Bb	11.7 ± 0.8 Abc	14.9 ± 1.3 Bb	-
P. virgatum	2009	7.3 ± 0.4 Ab	7.0 ± 0.3 Ab	8.5 ± 0.3 Aab	9.1 ± 0.5 Aab	12.1 ± 0.4 Aa	10.8 ± 0.4 Aab	10.9 ± 0.4 Aab
'Northwind'	2010	10.3 ± 0.4 Aab	10.1 ± 0.2 Aab	11.0 ± 0.4 Aab	11.4 ± 0.3 Aab	7.7 ± 0.4 Bb	11.2 ± 0.6 Aab	11.9 ± 0.4 Aa
	2011	9.3 ± 0.4 Aab	7.0 ± 0.7 Ab	8.4 ± 0.3 Aab	10.4 ± 0.5 Aab	10.4 ± 0.6 ABab	11.5 ± 0.5 Aa	10.7 ± 0.3 Aab
P. virgatum	2009	9.3 ± 0.8 Ad	19.3 ± 1.2 ABc	25.9 ± 1.2 Ab	31.0 ± 3.4 Aa	26.5 ± 2.5 Bb	29.8 ± 1.7 Bab	33.7 ± 2.7 Ba
'Prairie Fire'	2010	12.7 ± 0.7 Ad	21.6 ± 1.9 Ac	22.6 ± 1.1 Ac	23.5 ± 1.2 Bc	41.4 ± 2.3 Ab	49.7 ± 3.4 Aa	42.1 ± 4.3 Ab
	2011	12.9 ± 1.1 Ac	16.6 ± 1.0 Bc	24.4 ± 2.1 Ab	26.9 ± 2.4 Bb	39.6 ± 6.0 Aa	28.0 ± 3.2 Bb	38.0 ± 5.5 Aa
P. virgatum	2009	7.0 ± 0.8 Bc	15.0 ± 2.5 Bb	15.0 ± 1.4 Bb	20.2 ± 2.6 Ba	19.5 ± 2.9 Ba	15.4 ± 2.1 Cb	22.8 ± 3.6 Ba
'Red Sunset'	2010	12.8 ± 1.2 Ad	16.7 ± 1.0 ABcd	17.5 ± 0.9 Bc	18.3 ± 1.9 Bc	26.8 ± 2.1 Ab	31.0 ± 2.7 Aa	33.0 ± 5.6 Aa
	2011	16.1 ± 2.3 Ad	20.2 ± 1.6 Abc	23.5 ± 4.4 Aab	24.5 ± 2.9 Aa	21.8 ± 1.8 Bab	22.1 ± 2.2 Bab	16.9 ± 2.3 Ccd
P. virgatum	2009	12.5 ± 1.7 Bd	25.9 ± 2.9 Ac	31.9 ± 4.0 Ab	36.9 ± 3.0 Aa	39.6 ± 6.7 Ba	32.8 ± 3.6 Cb	36.5 ± 2.9 Ba
Ruby Ribbons™	2010	13.5 ± 1.1 Bf	22.3 ± 0.9 Ae	26.1 ± 1.1 Bde	29.9 ± 1.8 Bd	44.4 ± 2.4 Ac	51.7 ± 3.4 Ab	57.7 ± 6.5 Aa
	2011	18.5 ± 0.7 Ae	24.9 ± 1.0 Ad	31.7 ± 2.8 Ac	37.8 ± 3.0 Ab	38.9 ± 4.6 Bb	46.8 ± 6.1 Ba	32.6 ± 2.1 Bc
P. virgatum	2009	9.2 ± 1.0 Ad	22.2 ± 2.4 Ac	24.5 ± 3.3 Abc	26.3 ± 2.7 Bb	27.1 ± 3.1 Bab	28.0 ± 2.8 Bab	30.8 ± 2.4 Ba
'Shenandoah'	2010	9.6 ±0.6 Af	15.8 ± 0.5 Be	18.8 ± 0.7 Bde	21.8 ± 1.2 Cd	38.0 ± 2.7 Ac	44.7 ± 3.6 Ab	53.5 ± 4.6 Aa
	2011	12.9 ±1.1 Ad	13.9 ± 0.8 Bd	23.1 ± 1.9 Ac	32.4 ± 2.0 Ab	36.4 ± 2.2 Aa	29.6 ± 3.0 Bb	30.9 ± 2.1 Bb
P. advena	2009	25.8 ± 2.4 c	33.0 ± 2.6 b	42.8 ± 2.9 a	32.4 ± 2.0 b	28.1 ± 2.5 c	34.0 ± 2.2 b	34.9 ± 2.5 b

Table 2.2 (cont.)

Taxa	Year	Week						
		28	30	32	34	36	38	40
P. purpureum 'Prince'	2009	10.9 ± 0.9 c	14.6 ± 1.4 bc	19.7 ± 0.9 a	12.7 ± 0.6 bc	11.1 ± 0.5 c	14.8 ± 0.8 bc	16.4 ± 1.3 ab
S. scoparium Blue Heaven™	2009	8.8 ± 0.5 Ad	12.4 ± 1.2 Acd	17.0 ± 2.3 Ab	18.9 ± 1.7 ABb	26.1 ± 4.2 Aa	15.7 ± 1.5 Bbc	24.8 ± 3.0 Aa
	2010	9.6 ± 1.0 Ad	12.0 ± 1.1 Acd	16.9 ± 1.4 Ab	22.4 ± 3.2 Aa	15.8 ± 2.2 Bbc	23.1 ± 3.3 Aa	17.7 ± 1.2 Bb
	2011	11.6 ± 1.4 Ac	15.3 ± 2.4 Aab	11.4 ± 1.1 Bb	17.1 ± 2.2 Ba	16.2 ± 1.5 Ba	16.9 ± 1.9 Ba	17.7 ± 1.8 Ba
S. scoparium variegated	2009	5.6 ± 0.7 Ab	7.0 ± 0.4 Aab	9.8 ± 1.2 Aa	7.0 ± 1.2 Aab	6.8 ± 0.5 Bab	9.8 ± 1.4 Aa	7.5 ± 0.6 Bab
	2010	5.3 ± 0.5 Ab	4.7 ± 0.5 Ab	5.3 ± 0.5 Bb	5.3 ± 0.5 Ab	4.4 ± 0.5 Bb	7.3 ± 0.7 Ab	12.0 ± 1.6 Aa
	2011	7.3 ± 1.1 Abc	4.5 ± 0.3 Ac	6.2 ± 1.2 ABbc	7.7 ± 1.2 Abc	12.4 ± 1.8 Aa	8.9 ± 1.1 Aab	9.0 ± 0.9 ABab

Table 2.3. Correlations between anthocyanin or chlorophyll content ($\mu g \cdot cm^{-2}$) and average daily temperature (ADT, °C), total growing degree days (GDD, °C), average daily light integral (DLI, $mol \cdot m^{-2} \cdot d^{-1}$) and total irradiance for 10 grass taxa sampled every 2 weeks during summer and autumn (weeks 28 to 40) for 3 years, 2009-11. Average daily temperature and DLI are mean values for the 2-week interval preceding each leaf harvest. Total GDD and DLI are cumulative values from May 1 until each leaf harvest in each year.

Pigment	Weather parameter	Taxa				
		P. virgatum 'Northwind'	*P. virgatum* 'Prairie Fire'	*P. virgatum* 'Red Sunset'	*P. virgatum* Ruby Ribbons™	*P. virgatum* 'Shenandoah'
Anthocyanin ($\mu g \cdot cm^{-2}$)	ADT	-0.50	-0.61	-0.35	-0.62	-0.67
	Total GDD	0.59	0.88	0.73	0.86	0.86
	Avg DLI	-0.46	-0.79	-0.59	-0.71	-0.77
	Total DLI	0.57	0.83	0.59	0.82	0.81
Chlorophyll ($\mu g \cdot cm^{-2}$)	ADT	-0.26	0.62	0.05	0.57	0.64
	Total GDD	0.64	-0.90	-0.39	-0.85	-0.84
	Avg DLI	-0.40	0.75	0.27	0.62	0.72
	Total DLI	0.52	-0.85	-0.22	-0.75	-0.79

Table 2.3 (cont).

Pigment	Weather parameter	Taxa				
		S. scoparium Blue Heaven™	*S. scoparium* variegated	*I. cylindrica* 'Red Baron'	*P. advena*	*P. purpureum* 'Prince'
Anthocyanin ($\mu g \cdot cm^{-2}$)	ADT	-0.48	-0.61	-0.34	0.01	-0.11
	Total GDD	0.63	0.50	0.07	0.20	0.18
	Avg DLI	-0.55	-0.54	-0.08	-0.04	-0.10
	Total DLI	0.73	0.57	0.08	0.22	0.20
Chlorophyll ($\mu g \cdot cm^{-2}$)	ADT	0.41	-0.08	0.42	0.67	0.88
	Total GDD	-0.72	-0.29	-0.54	-0.81	-0.72
	Avg DLI	0.58	0.04	0.29	0.64	0.71
	Total DLI	-0.64	-0.10	-0.52	-0.82	-0.72

Table 2.4. Total chlorophyll content ($\mu g \cdot cm^{-2}$) for 10 grass taxa varying in foliar coloration. Leaf samples were harvested every 2 weeks (weeks 28-40) for three years (2009-11) to quantify seasonal variation in leaf pigmentation. For each taxa, means ($\pm$ SE) in each column followed by similar uppercase letters and means in each row followed by similar lowercase letters are not significantly different at $P<0.05$ (mean separation performed using Tukey's HSD).

Taxa	Year	Week						
		28	30	32	34	36	38	40
I. cylindrica	2009	15.2 ± 0.7 Aa	7.3 ± 0.1 Ab	7.1 ± 0.7 Ab	8.7 ± 1.2 Ab	4.4 ± 0.7 Ab	5.6 ± 0.7 Ab	6.4 ± 1.1 Ab
'Red Baron'	2010	5.7 ± 0.7 Ba	9.8 ± 1.4 Aa	7.3 ± 0.8 Aa	4.7 ± 0.5 Aa	4.0 ± 0.4 Aa	3.7 ± 0.5 Aa	3.9 ± 0.7 Aa
	2011	5.2 ± 0.8 Bb	10.7 ± 0.9 Aab	12.5 ± 0.5 Aa	6.6 ± 0.8 Aab	6.0 ± 0.7 Ab	6.3 ± 0.3 Ab	-
P. virgatum	2009	46.2 ± 2.6 Bde	40.0 ± 3.8 Ce	49.1 ± 2.6 Bd	44.3 ± 4.0 Cde	56.6 ± 4.0 Cc	78.1 ± 5.6 Aa	64.1 ± 3.0 Bb
'Northwind'	2010	58.0 ± 1.8 Ac	66.2 ± 1.5 Ab	68.6 ± 1.2 Ab	70.9 ± 1.8 Aab	71.1 ± 1.5 Aab	71.3 ± 2.2 Bab	75.7 ± 3.4 Aa
	2011	58.0 ± 2.4 Ab	46.5 ± 3.8 Bc	50.9 ± 1.8 Bc	58.3 ± 2.1 Bb	67.7 ± 2.3 Ba	69.9 ± 4.5 Ba	58.0 ± 2.3 Cb
P. virgatum	2009	41.6 ± 2.4 Aa	30.0 ± 2.6 Ab	18.9 ± 2.3 Acd	23.8 ± 1.8 Ac	17.2 ± 1.5 Ad	13.4 ± 1.9 Ad	14.1 ± 1.6 Ad
'Prairie Fire'	2010	29.4 ± 1.2 Ba	19.8 ± 1.2 Bbc	20.5 ± 1.6 Abc	24.2 ± 1.2 Aab	14.5 ± 1.9 Acd	10.9 ± 2.0 Ade	5.4 ± 0.9 Ae
	2011	31.4 ± 1.9 Ba	24.0 ± 3.5 ABb	22.3 ± 2.9 Abc	17.6 ± 2.9 Bcd	15.4 ± 2.5 Ade	12.3 ± 1.7 Ade	9.9 ± 1.7 Ae
P. virgatum	2009	31.4 ± 1.2 Aa	23.4 ± 0.9 Ab	23.2 ± 1.4 Abc	22.9 ± 2.8 CAc	20.2 ± 1.3 Abc	17.4 ± 1.2 Abc	17.2 ± 1.6 Ac
'Red Sunset'	2010	17.7 ± 1.4 Ba	17.8 ± 3.3 ABa	13.9 ± 1.9 Bab	10.0 ± 0.6 Bbc	6.6 ± 0.5 Bc	5.7 ± 1.6 Bc	6.9 ± 2.0 Bc
	2011	18.4 ± 2.0 Bab	12.6 ± 0.9 Bbc	12.0 ± 2.3 Bc	24.2 ± 1.4 Aa	23.3 ± 2.4 Aa	20.6 ± 2.9 Aa	23.3 ± 2.6 Aa
P. virgatum	2009	34.9 ± 2.7 Aa	26.1 ± 2.6 Bb	22.2 ± 2.2 Abc	24.4 ± 1.3 Ab	27.1 ± 3.4 Ab	17.0 ± 3.3 Ac	21.9 ± 3.1 Abc
Ruby Ribbons™	2010	27.7 ± 2.4 Ba	29.1 ± 1.8 ABa	26.1 ± 1.0 Aa	23.1 ± 1.7 Aa	13.3 ± 0.8 Bbc	8.3 ± 0.8 Bc	15.7 ± 2.5 Bb
	2011	27.3 ± 1.3 Bb	35.0 ± 1.2 Aa	21.4 ± 1.7 Ac	16.0 ± 2.1 Bcd	11.6 ± 1.0 Bd	15.2 ± 2.9 Acd	10.8 ± 2.2 Bd
P. virgatum	2009	31.6 ± 1.5 Aa	18.6 ± 1.5 Bb	17.1 ± 2.8 Ab	16.4 ± 1.8 Ab	20.4 ± 2.4 Ab	18.1 ± 2.3 Ab	14.5 ± 1.0 Ab
'Shenandoah'	2010	21.9 ± 1.1 Aa	20.5 ± 1.5 Ba	17.4 ± 1.2 Aab	14.2 ± 2.0 Abc	12.9 ± 0.7 Bbc	12.3 ± 1.2 Abc	9.8 ± 1.2 ABc
	2011	30.2 ± 1.3 Aa	29.0 ± 1.4 Aa	19.2 ± 1.3 Ab	17.2 ± 1.7 Abc	12.7 ± 2.6 Bc	12.1 ± 1.6 Ac	4.0 ± 1.2 Bd
P. advena	2009	47.8 ± 1.8 a	45.4 ± 0.8 a	48.1 ± 2.7 a	47.2 ± 1.2 a	37.5 ± 2.1 b	34.9 ± 1.4 b	38.6 ± 1.7 b

Table 2.4 (cont.)

Taxa	Year	Week						
		28	30	32	34	36	38	40
P. purpureum 'Prince'	2009	49.3 ± 1.6 ab	52.9 ± 3.2 a	52.5 ± 2.1 a	54.8 ± 6.4 a	40.0 ± 3.6 c	44.3 ± 3.2 bc	36.2 ± 3.6 d
S. scoparium Blue Heaven™	2009	48.1 ± 2.8 Aa	45.1 ± 1.7 Aa	50.8 ± 2.1 Aa	44.7 ± 2.3 Aa	33.9 ± 5.3 Ab	26.0 ± 4.1 Ac	27.0 ± 4.3 ABc
	2010	44.8 ± 2.7 Aa	34.2 ± 2.1 Bb	30.0 ± 1.7 Bbc	25.9 ± 4.5 Bcd	15.0 ± 1.5 Be	9.5 ± 2.8 Be	23.8 ± 1.3 Bd
	2011	46.5 ± 4.4 Aab	41.4 ± 3.5 Ab	49.4 ± 3.2 Aa	44.0 ± 2.8 Aab	34.4 ± 1.8 Ac	32.6 ± 3.0 Ac	32.1 ± 4.0 Ac
S. scoparium variegated	2009	17.1 ± 1.3 Aa	17.1 ± 1.8 Aa	17.3 ± 1.9 Aa	18.5 ± 2.0 Aa	10.7 ± 1.6 ABb	14.3 ± 1.6 Aab	15.0 ± 0.9 Aab
	2010	12.8 ± 0.8 Aa	14.6 ± 0.9 ABa	12.9 ± 0.7 ABa	11.2 ± 1.6 Ba	9.5 ± 0.7 Ba	8.7 ± 0.5 Aa	14.2 ± 1.6 Aa
	2011	12.4 ± 1.6 Aab	10.9 ± 1.6 Bab	9.5 ± 1.3 Bb	15.6 ± 1.4 ABab	16.7 ± 1.0 Aa	11.0 ± 0.7 Aab	13.8 ± 1.6 Aab

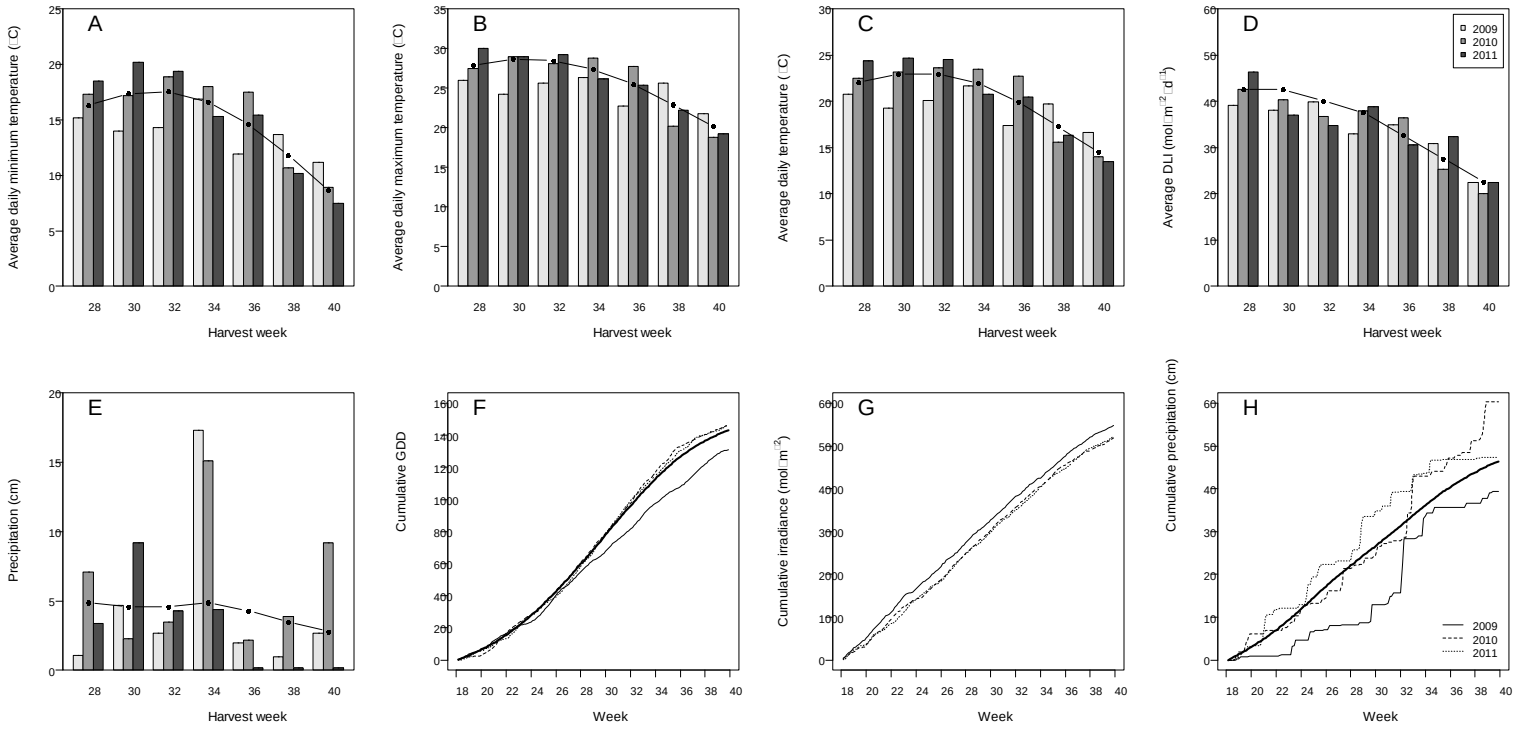

Fig. 2.1. Temperature, irradiance, and precipitation data for 2009-11. Weather data for each harvest week were for the 2 weeks prior to each harvest: A) average daily minimum temperature (°C), B) average daily maximum temperature (°C), C) average daily temperature (°C), D) average daily light integral (DLI, $mol \cdot m^{-2} \cdot d^{-1}$), and E) total precipitation (cm). The line plot overlaid on the graphs represents the 30-year (1971-2000) historical average for Minneapolis/St. Paul, MN. Historical average temperature and irradiance data are from NOAA (2012), and historical average DLI data are from maps developed by Korczynski et al. (2002). Cumulative weather data since May 1 (week 18) are shown, with the solid, bold line representing the 30-year historical average: F) total growing degree days (GDD, °C), G) total irradiance ($mol \cdot m^{-2}$), and H) total precipitation (cm).

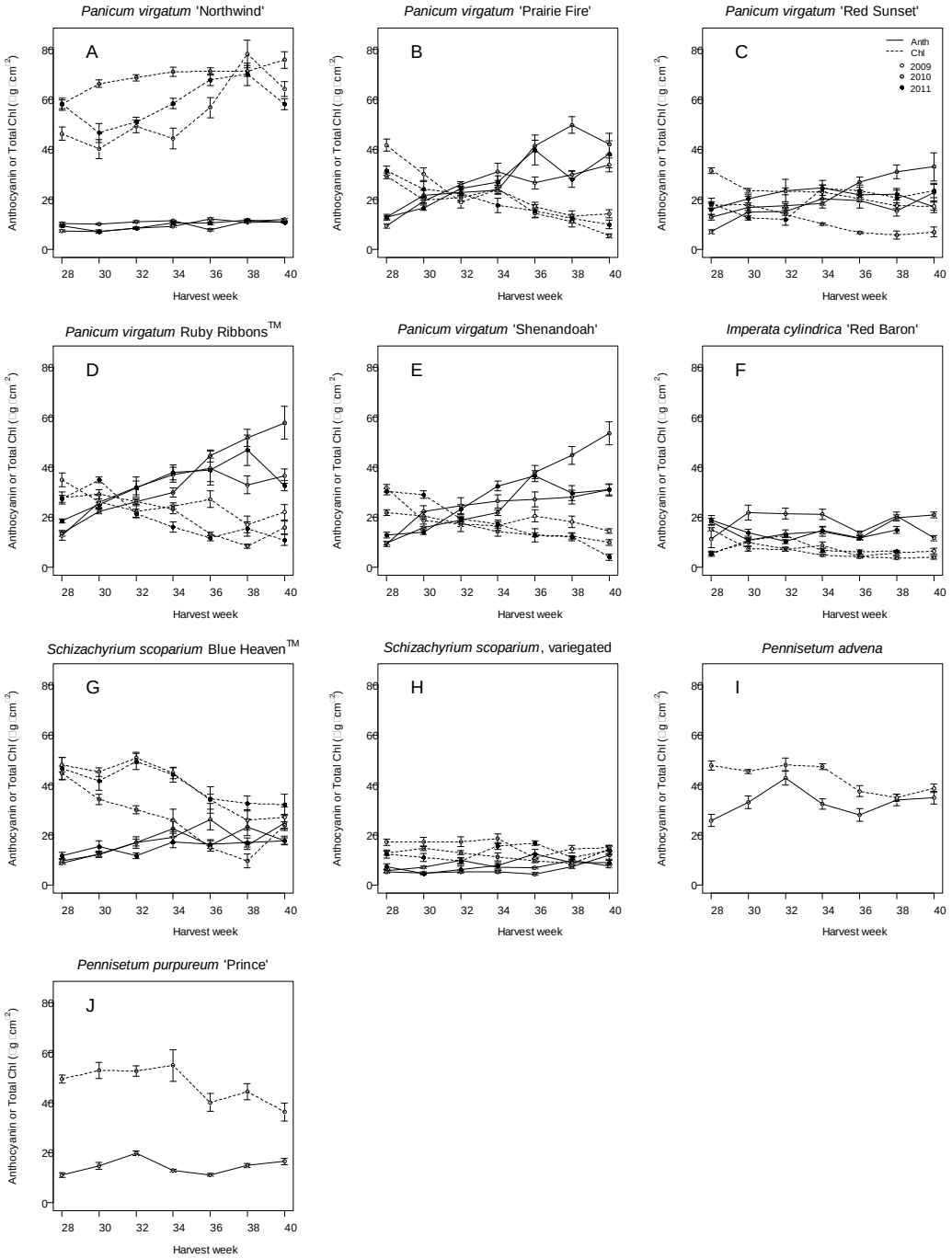

Fig. 2.2. Foliar anthocyanin and chlorophyll content (μg·cm^{-2}) of 10 grasses varying in leaf coloration. Leaves were harvested every 2 weeks (weeks 28 to 40) over three years (2009-11). Taxa: A) *Panicum virgatum* 'Northwind', B) *P. virgatum* 'Prairie Fire', C) *P. virgatum* 'Red Sunset', D) *P. virgatum* Ruby Ribbons™, E) *P. virgatum* 'Shenandoah', F) *Imperata cylindrica* 'Red Baron', G) *Schizachyrium scoparium* Blue Heaven™, H) variegated *S. scoparium*, I) *Pennisetum advena* (2009 only), and J) *Pennisetum purpureum* 'Prince' (2009 only).

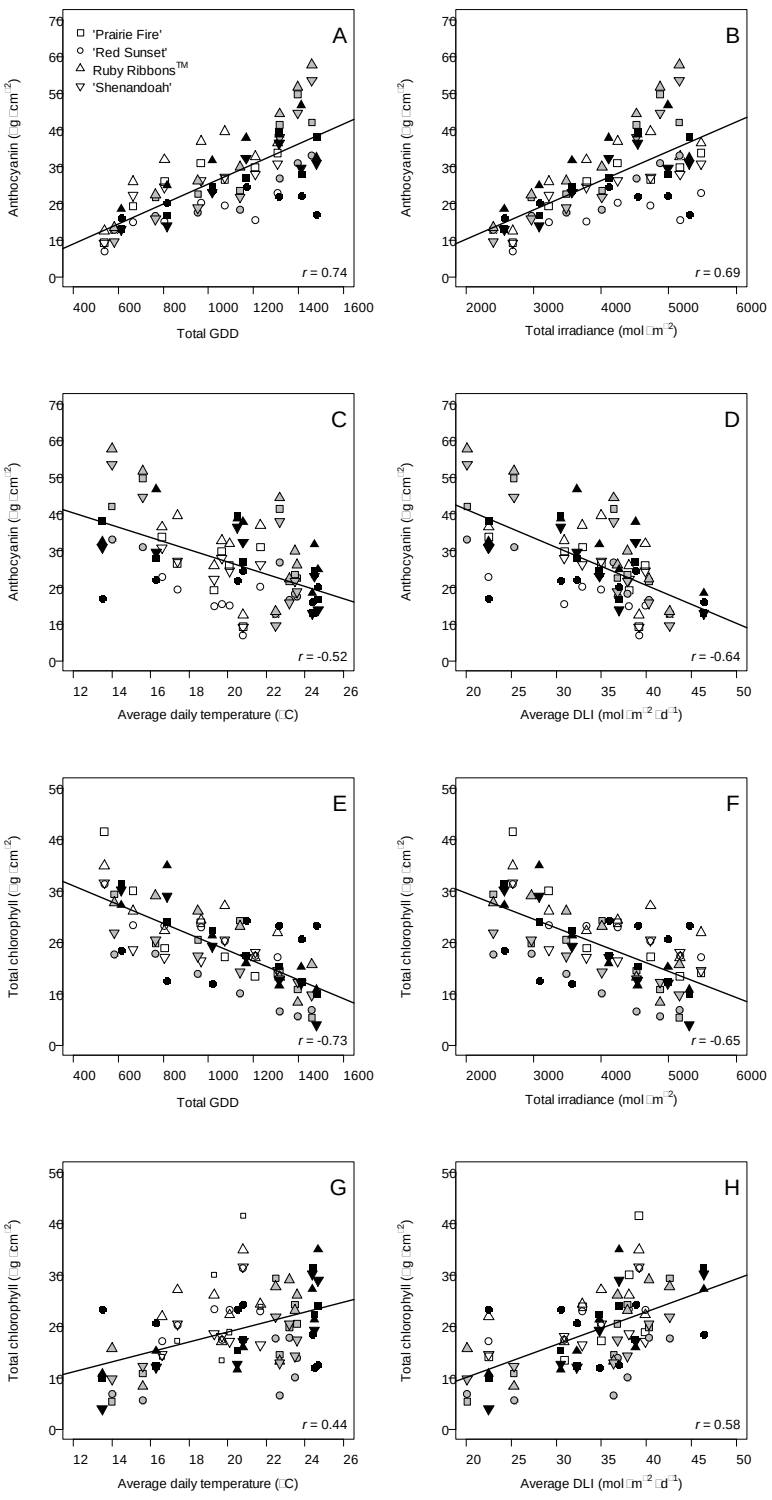
A
'Prairie Fire'
'Red Sunset'
Ruby Ribbons™
'Shenandoah'
Anthocyanin (□g□cm⁻²)
Total GDD
r = 0.74
B
Total irradiance (mol□m⁻²)
r = 0.69
C
Average daily temperature (□C)
r = -0.52
D
Average DLI (mol□m⁻²□d⁻¹)
r = -0.64
E
Total chlorophyll (□g□cm⁻²)
r = -0.73
F
r = -0.65
G
r = 0.44
H
r = 0.58

Fig. 2.3. Correlations between pigment concentration and environmental conditions for four anthocyanic *P. virgatum* cultivars across three years: A) anthocyanin and total growing degree days (GDD, °C), B) anthocyanin and total irradiance ($mol \cdot m^{-2}$), C) anthocyanin and average daily temperature (ADT), D) anthocyanin and average daily light integral (DLI, $mol \cdot m^{-2} \cdot d^{-1}$) , E) total chlorophyll and total GDD, F) total chlorophyll and total irradiance, G) total chlorophyll and ADT, and H) total chlorophyll and average DLI. Total GDD and irradiance were summed beginning May 1 each year, and ADT and DLI correspond to the 2 week interval prior to each harvest. Symbols: white = 2009, grey = 2010, and black = 2011. Cultivars: 'Prairie Fire' (■), 'Red Sunset' (●), Ruby Ribbons™ (▲), and 'Shenandoah' (▼).

Chapter 3

Solenostemon scutellarioides and *Panicum virgatum* Cultivars Vary in Photosynthetic Responses to Short-Term Irradiance, Carbon Dioxide, and Temperature Fluctuations

Photosynthesis was measured in nine red and green *Solenostemon scutellarioides* L. (Codd.) (coleus) and *Panicum virgatum* L. 'Heavy Metal' and 'RR1' Ruby Ribbons™ (switchgrass) cultivars over a range of irradiance, temperature, and CO_2 concentrations. Maximum photosynthetic rates (A_{max}) varied between red and green cultivars depending on how CO_2 uptake was expressed. A_{max} in coleus was similar in red and green leaves per unit leaf area, higher in red leaves per unit fresh weight or dry weight, and lower in red leaves per unit chlorophyll at saturating irradiance. A_{max} in switchgrass leaves at saturating irradiance was higher in green leaves per unit leaf area, fresh weight, and dry weight, and similar in red and green leaves per unit chlorophyll. No differences in the light compensation or light saturation points were observed between red and green leaves in either species, although cultivar differences were observed for the light saturation point in coleus. A_{max} at saturating CO_2 concentrations was similar in red and green coleus when quantified on an area, fresh weight, and dry weight basis, but higher in green leaves per unit chlorophyll. Likewise, A_{max} at saturating CO_2 concentrations was higher in green switchgrass leaves per unit leaf area, fresh weight, and dry weight, but lower in green switchgrass per unit chlorophyll. Photoprotection by anthocyanins was minimal at high irradiance or CO_2-limiting conditions. Effective quantum yield, electron transport

rate, and photochemical quenching were similar between red and green leaves in coleus and switchgrass. In response to temperature, red and green coleus had similar photosynthetic rates at 10 °C and 30 °C, but green leaves had higher photosynthetic rates than red leaves between 15 °C and 25 °C. In switchgrass, photosynthesis increased in both red and green leaves as temperature increased from 10 °C to 30 °C. Anthocyanins appeared to enhance photoprotection at lower temperatures in coleus, but not switchgrass. As temperature decreased from 20 °C to 10 °C, green coleus leaves had a greater decrease (80%) in the ratio of variable to maximum fluorescence (F_v/F_m) than red leaves (15%), but a similar decline was observed in red and green switchgrass leaves (36%).

Introduction

Anthocyanins are pigments in a group of secondary compounds known as flavonoids and are responsible for red and burgundy foliage coloration in many plant species (Chalker-Scott, 1999; Tanaka et al., 2008). Anthocyanins are localized in single or multiple cell layers in leaves and stems (Gould et al., 2002). They can accumulate in the adaxial epidermis (Burger and Edwards, 1996; Neill and Gould, 2003; Pietrini et al., 2002), the mesophyll (Choinski et al., 2003; Neill and Gould, 1999; Woodall et al., 1998), and the abaxial epidermis (Lee and Collins, 2001; Lee et al., 1979). In *Quintinia serrata*, leaf anthocyanins are distributed primarily in palisade and mesophyll cells, but can be in 15 of 16 possible cell layer combinations (Gould et al., 2000).

Anthocyanins can protect leaf photosynthesis by minimizing the severity of abiotic stresses, such as high irradiance, low temperature, and/or nutrient deficiency (Chalker-Scott, 1999; Close and Beadle, 2003). They are hypothesized to protect leaf photosynthesis by filtering excess light and/or scavenging free radicals (Neill and Gould, 2003), and their function may be related to localization within a leaf. For instance, anthocyanins present in the adaxial epidermis or in sub-epidermal cells are more likely to attenuate excess irradiation, and anthocyanins present in photosynthetic palisade or spongy mesophyll cells are more likely to function as free-radical scavengers (Neill and Gould, 2003).

Photoinhibition, a decline in photosynthetic rate, can occur when more photons of light are absorbed by a leaf than used in photochemistry (Krause, 1988). Such photoinhibition can occur under high irradiance in sun-adapted, non-stressed leaves or at lower irradiance in shade-adapted or stressed leaves, and it is most pronounced under low temperature (Demmig-Adams and Adams, 1992). In healthy, non-stressed plants, almost all photons of light absorbed by chloroplasts at low and moderate light intensities are directed towards photochemistry (photochemical quenching). As irradiance increases, the influx of photons available for photochemistry will eventually exceed the capacity of carbon fixation. At light saturation, photochemistry will be low, and excess light energy will be dissipated through non-photochemical quenching (primarily through heat dissipation via the xanthophyll cycle) or, unavoidably, through the generation of oxygen radicals.

When photoinhibition occurs, excess light energy is often dissipated by formation of oxygen radicals, which can damage membranes (Krause, 1988). These oxygen radicals may be quenched by antioxidants, including anthocyanins, before they cause damage to photosystems and thylakoid membranes (Krause, 1988; Neill and Gould, 2003). If irradiance increases above the capacity of photochemical and non-photochemical quenching mechanisms and free radical scavenging, then destructive processes will begin to take place (Demmig-Adams and Adams, 1992). Anthocyanins may 1) delay the onset or severity of photoinhibition by absorbing light before it reaches chloroplasts, or 2) mitigate the degree of photoinhibition by quenching oxygen radicals before they damage thylakoid membranes (Neill and Gould, 2003).

Attenuation of light by anthocyanins can lower photosynthetic rates at low irradiance levels, but may result in higher photosynthetic rates at high irradiance (or whenever incoming light exceeds the capacity of the photosystems) when their benefit offsets the potential reduction in photosynthesis due to photoinhibition. Lower photosynthetic rates in red leaves have been observed in *Quintinia serrata* at saturating irradiance levels (Gould et al., 2002), young *Syzygium* species (Woodall et al., 1998), *Cotyledon orbiculata* leaves (Barker et al., 1997), and *Brachystegia spiciformis* (Tuohy and Choinski, 1990). However, similar photosynthetic rates were observed in red and green coleus leaves when exposed to red or white light (Burger and Edwards, 1996), *Prunus persica* L. Batsch leaves (Marini, 1986), and *Zea mays* leaves (Pietrini et al., 2002). Higher photosynthetic rates were observed in red coleus leaves compared to green leaves following a 3 h UV exposure (Burger and Edwards, 1996), and in red *Triolena*

hirsuta and *Begonia pavonina* leaves (Gould et al., 1995). Conflicting observations in differences in photosynthetic rates between red and green leaves may be due to differences in leaf anthocyanin concentration, anthocyanin distribution across the leaf surface or within a leaf, as well as ambient environmental conditions at which photosynthesis was measured.

The objective of our research presented here was to compare net photosynthetic rates and chlorophyll fluorescence of red and green leaves of *Solenostemon scutellarioides* L. (Codd) (coleus; C_3 plant) and *Panicum virgatum* L. (switchgrass; C_4 plant) in response to short-term fluctuations in irradiance, CO_2, and temperature. The experiment was conducted to determine if anthocyanins provided a photosynthetic advantage (higher rates of net photosynthesis, P_n, or greater photosynthetic efficiency) in these two species across a range of irradiances, CO_2 concentrations, or temperatures.

Materials and Methods

Plant culture

Coleus 'Versa Burgundy to Green', 'Versa Crimson Gold' and 'Versa Lime' seed (PanAmerican Seed, Ball Horticultural Company, West Chicago, IL) were sown on 10 July 2010, one seed per cell, in a 128-count cell tray filled with soilless media (Metro Mix 200; SunGro Horticulture, Bellvue, WA). Sown trays were placed under intermittent mist for 14 d, until most seedlings germinated and cotyledons were horizontal to the media surface. Seedlings were moved to a greenhouse and grown under

ambient irradiance [daily light integral (DLI) was 14.3 ± 2.3 mol·m^{-2}·d^{-1} (mean ± $_{SD}$)], plus 2 µmol·m^{-2}·s^{-1} lighting from incandescent bulbs nightly from 2200-0200 $_{HR}$, and greenhouse temperatures were 28.3 ± 2.5 °C day/24.7 ± 2.3 °C night (mean ± $_{SD}$). Plants received night interruption lighting to keep them vegetative. Seedlings were transplanted six weeks after sowing (21 Aug. 2010) into 10 cm diameter square pots (volume = 815 cm^3) filled with LC-8 soilless media (SunGro Horticulture, Bellvue, WA), and the primary shoot was pruned to one node 10 d after transplant.

Cuttings of coleus 'Big Red Judy', 'LifeLime', 'Royal Glissade', and 'Sedona' (Pleasant View Gardens, Loudon, NH), and red and green-leaved selections of 'Twist and Twirl' (courtesy of D.G. Clark, University of Florida, Gainesville, FL) were harvested on 24 July 2010 from stock plants maintained in 16.5 cm diameter pots (volume = 1.3 L) in a greenhouse (University of Minnesota, St. Paul, MN). Greenhouse temperatures during cutting production were 28.6 ± 3.1 °C day/24.4 ± 3.2 °C night (mean ± $_{SD}$) and the mean DLI was 15.2 ± 2.4 mol·m^{-2}·d^{-1}. Plants received night interruption lighting (2 µmol·m^{-2}·s^{-1} from incandescent bulbs nightly, 2200-0200 $_{HR}$) to keep them vegetative. Cuttings were pruned to two nodes, inserted into a 50-count cell tray (volume = 110 cm^3) filled with LC-8 soilless media, and placed under intermittent mist (5 s every 15 min) for 7 d until root formation occurred. Rooted cuttings were then moved into the greenhouse and transplanted three weeks later (21 Aug. 2010) into 10 cm square pots filled with LC-8 soilless media. Plants were pruned to one node 10 d after transplant.

Switchgrass 'Heavy Metal' and 'RR1' Ruby Ribbons^{TM} (Emerald Coast Growers, Pensacola, FL) plants were sectioned into divisions on 21 Aug. 2010 and planted. Each

division contained five to seven young tillers (< 5 cm tall) once mature, flowering stems were removed. Divisions were transplanted into 10 cm diameter square pots diameter pots filled with SB500, a high-porosity soilless media (SunGro Horticulture, Bellvue, WA).

Coleus and switchgrass were watered as needed and fertilized weekly with 14.3 mM N from a 20N-4.4P-16.6K fertilizer (Peter's 20-10-20; The Scott's Co., Marysville, OH). A granular application of imidacloprid (Marathon; OHP, Inc., Mainland, PA) was applied at a rate of 1 $g \cdot pot^{-1}$ two weeks after transplantion for systemic insect control. Greenhouse temperatures between transplanting and the start of treatments were 27.5 ± 2.7 °C day/24.0 ± 2.5 °C night and mean DLI was 14.2 ± 2.3 $mol \cdot m^{-2} \cdot d^{-1}$. Four weeks after transplanting (18 Sept. 2010), coleus and switchgrass plants were moved into a growth chamber (3.3 m^2; Environmental Growth Chambers, Chagrin Falls, OH) maintained at a constant 20 ± 1 °C leaf temperature, a 16 h photoperiod (0600-2200 $_{HR}$), 50% relative humidity, and 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ irradiance (DLI = 17.3 $mol \cdot m^{-2} \cdot d^{-1}$) provided with metal halide lamps. Experiments began 7 d later after plants had an opportunity to acclimate to the growth chamber environment.

Photosynthesis response curves

Photosynthetic measurements were collected between 25 Sept. and 30 Oct. 2010 using a portable photosynthesis system (LI6400XT; LI-COR Biosciences, Inc., Lincoln, NE). A leaf chamber fluorometer (6400-40; LI-COR Biosciences, Inc., Lincoln, NE) was used to simultaneously measure gas exchange and chlorophyll fluorescence. Actinic

irradiance was supplied by a combination of red and blue light-emitting diodes (LEDs) in the fluorometer, with peaks at 630 nm and 470 nm, respectively (LI-COR, 2008). Ambient conditions within the leaf chamber matched growth chamber conditions except for the parameter of interest. Cuvette constants were as follows: 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ irradiance (10% blue light), 20 °C leaf temperature, 380 ppm reference CO_2 (μmol $CO_2 \cdot mol^{-1}$ air), 400 $\mu mol \cdot s^{-1}$ air flow, and ambient relative humidity (0.44 ± 0.05 for irradiance response curves, 0.56 ± 0.07 for CO_2 response curves; mean ± $_{SD}$).

Photosynthetic data were collected on fully-expanded, mature leaves (n=5). On coleus, one leaf at the fourth node below the shoot apex was selected from each plant. On switchgrass, two leaves from vegetative stems were positioned adjacent to each other within the cuvette to fill the measurement area (2 cm^2). Switchgrass leaves were of similar size and location so that they would be of similar age and photosynthetic capacity.

For irradiance response curves, each leaf (or pair of leaves) was positioned in a dark adapting clip (9964-091; LI-COR Biosciences, Inc., Lincoln, NE) for 15 min to allow it to dark adapt, then dark-adapted leaf fluorescence was measured (for calculation of F_v/F_m). The leaf was then illuminated with 500 $umol \cdot m^{-2} \cdot s^{-1}$ irradiance for 5 min, then increased up to 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ over a 10 min period. Resposes to irradiance were measured from high to low irradiance, from 2000 to 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Data were recorded once stability parameters were met, with minimum and maximum wait times of 2 and 3 min, respectively. Stability parameters were defined for photosynthesis, intercellular CO_2 concentration (C_i), stomatal conductance, and fluorescence. Values were calculated for net photosynthesis (A_n), maximum quantum yield (F_v/F_m), effective quantum yield

(Φ_{PSII}), electron transport rate (ETR), photochemical quenching (qP), and non-photochemical quenching (qN and NPQ).

The protocol for photosynthetic CO_2 response curves was the same as for the irradiance response curves, except irradiance was held constant and the reference CO_2 concentration was adjusted. Reference CO_2 concentration started at 400 μmol·mol^{-1}, was incrementally decreased to 50 μmol·mol^{-1}, returned to 400 μmol·mol^{-1} to minimize stomatal closure, then increased up to 1400 μmol·mol^{-1} (50 μmol·mol^{-1}, 100 to 1000 μmol·mol^{-1} at increments of 100 μmol·mol^{-1}, then 1200 and 1400 μmol·mol^{-1}). Sample and reference infrared gas analyzers (IRGAs) were matched at each reference CO_2 concentration to minimize drift between the IRGAs as CO_2 concentration varied. The minimum and maximum wait times at each CO_2 concentration were 1 min and 2 min, respectively.

For temperature response curves, photosynthesis and leaf fluorescence measurements were collected during the morning and early afternoon, with wait times of 2 to 4 min for photosynthesis to stabilize. Initial measurements were collected at 20 °C (ambient chamber temperature). The growth chamber temperature was adjusted following completion of the measurements, providing plants 16 h to acclimate to a new temperature before data were collected. Chamber temperature was increased to 25 °C and 30 °C before being returned to 20 °C for 1 d, then lowered to 15 °C then 10 °C. Measurements collected at 20 °C at the start and middle of the experiment were compared to ensure that there were no carry-over effects of the higher temperature exposures, and they were found to be similar.

Pigment quantification

Two leaf discs, one for anthocyanin and one for chlorophyll content, were sampled from one mature leaf per plant. Each leaf disc (0.3 cm^2) was placed in a 1.7 mL microcentrifuge tube containing either 1 mL of 99:1 (v:v) methanol:HCl (for anthocyanin extraction) or 95% ethanol (for chlorophyll extraction). Two tungsten carbide beads were added to each tube containing a switchgrass leaf disc. Switchgrass leaf tissue was homogenized in a Mixer Mill (MM 300; Qiagen, Valencia, CA), then placed in the dark at 4 °C for 18 h. Coleus leaf discs were transferred directly into cold storage without homogenization of the leaf tissue. Following cold storage, switchgrass samples were centrifuged (model 5714R; Eppendorf, Hamburg, Germany) at 4 °C for 10 min at 10,000 *g*. A 300 µL aliquot from each microcentrifuge tube was pipeted into a 96-well plate and absorbance was measured using a plate reader with monochromator optics (SpectraMax 190; Molecular Devices, Sunnyvale, CA). Maximum absorbance for anthocyanin was 530 nm for all species and cultivars (Fig. 3.1). Chlorophyll was measured at 649 nm and 665 nm. Absorbance values were normalized to a 1 cm pathlength and 1 cm^2 leaf area. Anthocyanin content was calculated as cyanidin-3-glucoside (Siegelman and Hendricks, 1958), and chlorophyll was calculated using equations published by Wintermans and De Mots (1965).

Experimental design and data analysis

The experimental design was a randomized complete block design. Data were analyzed as a two-way factorial with repeated measures because for each response curve, an individual leaf was subjected to a range of temperatures, irradiances, or CO_2 concentrations. There were five plants of each cultivar per treatment. Analysis of variance was conducted separately for each species using PROC MIXED in SAS (SAS 9.3, Cary, NC) with a repeated statement. Mean separation was conducted using Tukey's HSD (α=0.05) for sources of variation significant at P<0.05. In coleus, a subset of cultivars [three red (Big Red Judy', 'Twist and Twirl' red, and 'Versa Crimson Gold') and three green ('LifeLime', 'Twist and Twirl' green, and 'Versa Lime')] was analyzed to determine the impact of leaf color on photosynthesis and chlorophyll fluorescence. Other cultivars were excluded because their coloration was not uniformly red or green across the leaf surface.

Photosynthesis irradiance (A-I) and CO_2 (A-C_i) response curves were modeled using Photosyn Assistant (Dundee Scientific; Dundee, Scotland), based on equations published by Prioul and Chatier (1977) and Olsson and Leverenz (1994). A-I curves allowed for the estimation of important leaf photosynthetic characteristics, including the light compensation point (LCP, irradiance at which $A_n = 0$), the light saturation point (LSP, irradiance at which A_{max} is attained), maximum photosynthetic rate (A_{max}), and the apparent quantum efficiency (ϕ or AQE, the slope of the linear portion of an A-I curve). A-C_i curves allowed for the estimation of the intercellular CO_2 (C_i) compensation point, $A_{max,}$ and the carboxylation efficiency (CE). Individual A-I and A-C_i curves were

developed for each plant. Equation parameters of interest were analyzed by analysis of variance (randomized complete block design; n=5) and mean separation was conducted using Tukey's HSD (α=0.05).

Results

Irradiance

A-I curves

The LCP varied from 11 to 31 µmol·m^{-2}·s^{-1} in coleus cultivars and was 17 and 26 µmol·m^{-2}·s^{-1} in switchgrass Ruby Ribbons^{TM} (red leaves) and 'Heavy Metal' (green leaves), respectively (Table 3.1), but did not vary with leaf color (P = 0.46 for coleus, 0.09 for switchgrass). The LSP for coleus cultivars varied between 166 and 425 µmol·m^{-2}·s^{-1}, and it was 196 µmol·m^{-2}·s^{-1} in Ruby Ribbons^{TM} and 237 µmol·m^{-2}·s^{-1} in 'Heavy Metal' switchgrass. The LSP differed with cultivar in coleus but not with leaf color in either species (Table 3.1). AQE (based on incident irradiance) in coleus ranged between 0.02 ('Sedona') and 0.06 ('Twist and Twirl' red and 'Versa Burgundy to Green'), and differences in AQE were significant for cultivar but not leaf color in coleus. In switchgrass, however, the green-leaved 'Heavy Metal' had a significantly higher AQE (0.06) than the red-leaved Ruby Ribbons^{TM} (0.03) (P = 0.01).

Maximum net photosynthetic rates (A_{max}) ranged from 6.3 to 12.6 µmol CO_2·m^{-2}·s^{-1} among coleus cultivars. A_{max} varied by cultivar and was not consistent with leaf color (Table 3.1). When A_{max} was calculated on a fresh or dry weight basis, however, red

coleus leaves had higher photosynthetic rates than green leaves (Table 3.2). When A_{max} was calculated on a per unit chlorophyll basis, green coleus leaves had higher photosynthetic rates than red leaves (21.7 µmol $CO_2 \cdot g^{-1}$ chl$\cdot s^{-1}$ in green leaves and 16.0 $CO_2 \cdot g^{-1}$ chl$\cdot s^{-1}$ in red leaves, excluding 'Sedona'; Table 3.2). The higher A_{max} per unit chlorophyll in red coleus leaves is likely due to the fact that red coleus leaves generally were thinner and had more chlorophyll per unit area than the green leaves (Table 3.3). Almost the exact opposite was observed in switchgrass. 'Heavy Metal' had a higher A_{max} per unit area than Ruby Ribbons™ (12.4 and 5.6 µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$, respectively; Fig. 3.2B) and per unit fresh and dry weight, but a similar A_{max} per unit chlorophyll (Table 3.2). While both switchgrass cultivars had similar leaf fresh weights, 'Heavy Metal' leaves contained three times more chlorophyll than Ruby Ribbons™ leaves (Table 3.3).

Chlorophyll fluorescence

Similar trends in chlorophyll fluorescence were observed in coleus and switchgrass. Electron transport rate (ETR) and non-photochemical quenching (qN and NPQ) increased as irradiance increased, while effective quantum yield (Φ_{PSII}) and photochemical quenching (qP) decreased as irradiance increased (Fig. 3.3).

The effect of leaf color was not significant in coleus for any of the variables, and differences could be attributed to the interaction between cultivar and irradiance (Appendix C). At low irradiances ($\leq$ 300 µmol$\cdot m^{-2} \cdot s^{-1}$) coleus cultivars had similar values of Φ_{PSII}, qP, and ETR at each irradiance measured (Fig. 3.3A, C, and E). As irradiance increased, cultivars differed in the magnitude of their response but not in rank

order. At 0 μmol·m^{-2}·s^{-1} *PAR*, Φ_{PSII} in coleus was between 0.71 and 0.79 and declined to between 0.07 and 0.14 at 2000 μmol·m^{-2}·s^{-1} *PAR* (Fig. 3.3A). A greater than 2-fold difference in ETR was observed between coleus cultivars at saturating irradiances (Fig. 3.3C). Values of qP decreased from 1.0 at 0 μmol·m^{-2}·s^{-1} to less than 0.3 at 2000 μmol·m^{-2}·s^{-1} (Fig. 3.3E), and NPQ increased from 0 μmol·m^{-2}·s^{-1} to approximately 500 μmol·m^{-2}·s^{-1} *PAR* (Fig. 3.3G), then maintained a constant rate at higher irradiances.

In switchgrass, values of Φ_{PSII}, ETR, and qP were similar in the two cultivars (Fig. 3.3B, D, and F). Effective quantum yield (Φ_{PSII}) decreased from 0.65 to 0.06 in 'Heavy Metal' and from 0.62 to 0.05 in Ruby Ribbons^{TM} as irradiance increased from 0 to 2000 μmol·m^{-2}·s^{-1}, respectively. These values were lower than those observed in coleus at comparable irradiances. Values of qP were comparable to those observed in coleus at 2000 μmol·m^{-2}·s^{-1} (0.20 and 0.16 in 'Heavy Metal' and Ruby Ribbons^{TM}, respectively), but ETR was lower in switchgrass than coleus. NPQ differed between cultivars, but only at low irradiance; Ruby Ribbons^{TM} had higher NPQ than 'Heavy Metal' at irradiances at or below 100 μmol·m^{-2}·s^{-1} (Fig. 3.3H).

CO_2

A-C_i photosynthetic response curves

The internal CO_2 (C_i) compensation point in coleus leaves ranged from 23 to 60 μmol·mol^{-1} (Table 3.4), with differences associated with cultivar rather than leaf color. The C_i compensation point in switchgrass, a C_4 species, was lower, approximately 0 μmol·mol^{-1} for both cultivars. The carboxylation efficiency (CE, a measure of the unit

increase in photosynthesis per unit increase in CO_2) was lower in coleus than in green (but not red) switchgrass (Table 3.4). Average CE tends to be higher in C_4 plants due to their unique anatomy and ability to concentrate and fix CO_2 in bundle sheath cells. However, this efficiency was not evident in the red switchgrass Ruby Ribbons™.

A_{max} in switchgrass (13.3 and 11.8 µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ in 'Heavy Metal' and Ruby Ribbons™, respectively) was similar to the lower range in A_{max} observed in coleus (which varied between 12.3 and 24.5 µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$). A_{max} in coleus was not different between red and green leaves per unit leaf area (Fig. 3.4A), but green leaves had a higher A_{max} per unit fresh and dry weight (data not shown) and per unit chlorophyll (Fig. 3.4C). While there was a trend for A_{max} in switchgrass to be slightly higher in 'Heavy Metal' on a leaf area basis (Fig. 3.4B) and higher in Ruby Ribbons™ on a per unit chlorophyll basis (Fig. 3.4D), it was not significant due to a small sample size (n=5) and variability within each cultivar.

A-C_a photosynthetic response curves

The external CO_2 (C_a) compensation point was similar to the C_i compensation point in both species, ranging from 15 to 57 µmol·mol^{-1} in coleus and approximately 0 µmol·mol^{-1} in switchgrass. The highest rates of photosynthesis were observed at ≥ 900 µmol·mol^{-1} C_a in both species (Fig. 3.4E-H). Switchgrass 'Heavy Metal' had a higher photosynthetic rate than Ruby Ribbons™ per unit leaf area (Fig. 3.4F), but Ruby Ribbons™ had a higher A_{max} than 'Heavy Metal' per unit chlorophyll (Fig. 3.4H). The photosynthetic rate at A_{max} was 1.7 and 1.9-fold greater than A_n at ambient CO_2 (400

μmol·mol^{-1}) in 'Heavy Metal' and Ruby Ribbons^{TM}, respectively, and A_{max} in coleus was between 1.7 and 2.2-fold greater than P_n at ambient CO_2. This indicates that both species, at least in the short-term, could potentially double their net carbon uptake when grown at elevated CO_2.

Chlorophyll fluorescence

ETR, Φ_{PSII}, and qP increased in both species as CO_2 increased (Fig. 3.5A-F). No additional increases were observed above 600 μmol·mol^{-1} in switchgrass or above 700 μmol·mol^{-1} in coleus. NPQ decreased as CO_2 increased (Fig. 3.5G and H), with no additional decreases observed above 600 μmol·mol^{-1} in both species. Leaf color did not impact Φ_{PSII}, ETR, or qP in either species. Green coleus leaves had higher rates of NPQ than red leaves (0.66 in green leaves and 0.58 in red leaves, pooled across CO_2 concentration), but red switchgrass leaves had higher NPQ values than green leaves (0.99 in green leaves and 1.56 in red leaves pooled across CO_2 concentration).

Temperature

Photosynthesis

Net photosynthesis increased in coleus and switchgrass as temperature increased from 10 °C to 30 °C. No interaction between leaf color and temperature in coleus was observed when photosynthesis was expressed on a unit area or fresh weight basis (data not shown), but differences were evident when expressed on a per unit chlorophyll basis. Photosynthesis in red coleus leaves increased as temperature increased from 10 °C to 30

°C, while photosynthesis in green leaves increased from 10 °C to 25 °C before declining at 30 °C (Fig. 3.6A). At the lowest and highest temperatures (10 °C and 30 °C), red and green coleus leaves had similar rates of photosynthesis, but at the intermediate temperatures (15 °C to 25 °C), green leaves had higher rates of photosynthesis than red leaves. In contrast, there was no interaction between leaf color and temperature in switchgrass; both cultivars responded similarly. Average photosynthetic rate for switchgrass gradually increased, from 1.1 µmol $CO_2 \cdot g^{-1}$ chl$\cdot s^{-1}$ at 10 °C to 2.1 µmol $CO_2 \cdot g^{-1}$ chl$\cdot s^{-1}$ at 30 °C (pooled across cultivar).

Stomatal conductance was lowest at 10 °C in all coleus cultivars and relatively constant from 15 °C to 30 °C. However, stomatal conductance was low in 'LifeLime', a green cultivar, at 30 °C. This may partially explain why the average photosynthetic rate in green leaves declined at 30 °C, but another green cultivar ('Versa Lime') with decreased photosynthesis from 25 °C to 30 °C, had similar stomatal conductance between the two temperatures.

Chlorophyll fluorescence

Maximum quantum yield, F_v/F_m, decreased as temperature decreased below 20 °C (Fig. 3.6B). As temperature decreased, red and green switchgrass leaves showed similar declines in F_v/F_m. Values were similar at 20 °C and higher, but declined from 0.66 at 20 °C to 0.59 at 15 °C, and 0.44 and 10 °C, respectively. Red and green coleus leaves had similar F_v/F_m values at temperatures ≥ 20 °C, but green leaves had a larger decline in F_v/F_m as temperature decreased to 15 °C and then 10 °C. Red coleus leaves declined

from 0.81 at 20 °C to 0.76 at 15 °C, then 0.69 at 10 °C, while green leaves declined from 0.79 at 20 °C to 0.65 at 15 °C, then down to 0.44 at 10 °C (Fig. 3.6B).

Effective quantum yield (Φ_{PSII}) decreased as temperature decreased in both species (Fig. 3.6C). In coleus, Φ_{PSII} was influenced by temperature, but not leaf color, and decreased from 0.48 at 30 °C to 0.11 at 10 °C. In switchgrass, red and green leaves had a similar Φ_{PSII} at 10 °C, but 'Heavy Metal' increased more as temperature increased than did Ruby Ribbons™. A similar pattern was observed for ETR (Fig. 3.6D).

Values of qP decreased as temperature decreased in both species (Fig. 3.6E). In coleus, there was no difference between red and green leaves at each temperature. On average, qP decreased from 0.73 at 30 °C to 0.25 at 10 °C. No difference between qP in switchgrass cultivars was observed at temperatures between 10 °C and 25 °C; at 30 °C, 'Heavy Metal' was greater than Ruby Ribbons™ (0.83 and 0.65, respectively).

NPQ at each temperature was similar between red and green coleus leaves but not between switchgrass leaves. At 10 °C, both 'Heavy Metal' and Ruby Ribbons™ had similar values. as NPQ increased in both switchgrass cultivars from 10 to 25 °C the difference between the two cultivars increased in magnitude as temperature increased. NPQ declined in both from 25 °C to 30 °C (Fig. 3.6F).

Discussion

Anthocyanic leaves absorb more light than green leaves (Pietrini et al., 2002), especially in the green region of the visible spectrum, and a similar pattern exists in

acidified anthocyanic extracts (Neill and Gould, 2003). In our study, absorbance spectra of red leaf extracts in acidified methanol showed increased absorbance relative to green leaves, between 450 nm and 600 nm in coleus and between 450 nm and 580 nm in switchgrass (Fig. 3.1). Since we wished to simultaneously measure gas exchange and leaf fluorescence on the same leaf area, we were limited to using a leaf chamber containing blue and red LEDs (peaks at 470 nm and 630 nm, respectively) as the light source. Anthocyanins do not absorb much light at 630 nm, and at this wavelength, they may provide protection as an antioxidant in leaves but not as a light attenuator. However, anthocyanins do absorb some light at 470 nm (in our acidified methanol solutions, absorbance was approximately 30% to 50% of maximum absorbance at 530 nm) and may provide protection as either a light attenuator or antioxidant. Blue light was limited to 10% of the total light output at 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and that ratio was maintained at all irradiance intensities for consistency.

Photosynthetic responses to irradiance in our species may have been different had a different light source been used. For example, Burger and Edwards (1996) observed that red and green coleus leaves exposed to either white or red light had similar photosynthetic rates, whereas red coleus leaves exposed to green light had lower photosynthetic rates than green leaves. This was attributed to the capacity of anthocyanins to filter green light, decreasing the actual total *PAR* available for photosynthesis in underlying cells. In our study, A_{max} ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$) varied between coleus cultivars following exposure to red and blue light but red-leaved cultivars were not

consistently higher or lower than green-leaved cultivars, which coincides with results reported by Burger and Edwards (1996).

In *Zea mays*, red leaves absorbed more light than green leaves (89% vs. 80% *PAR*, respectively) but Pietrini et al. (2002) calculated only 61% of incident *PAR* in red leaves reached photosynthetic cells due to absorption by anthocyanins. Anthocyanins in coleus and switchgrass are present in epidermal cells (personal observation). Therefore, actual light absorption by chloroplasts in red leaves in our experiment was likely lower than that for chloroplasts in green leaves due to some light absorption by anthocyanins in the blue region, but it was not as reduced as would be anticipated under green light. If anthocyanins in our species provided some photoprotection under red and blue light, then it is likely that they could provide even greater protection when exposed to white or green light.

Anthocyanins did not influence the LCP or LSP in either species. LCPs were less than 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for both species and minimally affected by leaf color. LSPs were between 166-425 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in coleus and 196-237 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in switchgrass and were minimally affected by leaf color in either species. LCPs and LSPs tend to be higher in plants adapted to full sunlight (Lambers et al., 2008). Coleus is a C_3 tropical plant native to southeast Asia (Hanelt, 2001) and typically considered a shade-adapted plant. Recently, coleus breeding programs have selected and released cultivars adapted to full sun. All coleus cultivars in this experiment were recommended for partial- to full-sun light. In contrast, switchgrass is a native North American grass adapted to full sun. Interestingly, both species had similar LCPs and LSPs. This may be because plants were

acclimated to 300 µmol·m^{-2}·s^{-1} for 1 week before data collection. In another study, following acclimation to 300 µmol·m^{-2}·s^{-1} in a growth chamber, 13 herbaceous ornamental species light-saturated at 200-500 µmol·m^{-2}·s^{-1} regardless of place of origin (Boldt et al., 2011b).

On a leaf area basis (µmol CO_2·m^{-2}·s^{-1}), A_{max} varied almost 2-fold amongst coleus cultivars, but differences were associated with cultivar and not leaf color (Table 3.1). For instance, if one red and one green cultivar were randomly selected from the nine evaluated in this experiment and compared, it is possible to conclude a red cultivar had a higher A_{max} and the next time, with another comparison, conclude that a green cultivar had a higher A_{max}. The result that red and green leaves did not differ in A_{max} across a range of cultivars concurs with Burger and Edwards (1996), who concluded that there were no differences in photosynthetic rates between red and green-leaved coleus exposed to white or red light. Minimal differences in photosynthesis between red and green leaves have also been found for *Prunus persica* (Marini, 1986) and *Zea mays* (Pietrini et al., 2002).

In switchgrass, the green-leaved 'Heavy Metal' had a higher A_{max} per unit leaf area than the red-leaved Ruby Ribbons™ (Table 3.2). This concurs with results published for *Quintinia serrata*, in which green leaves had a 23% higher photosynthetic rate than red leaves at saturating irradiances (Gould et al., 2002), and *Brachystegia spiciformis* (Tuohy and Choinski, 1990). In addition, Woodall et al. (1998) reported that immature red leaves from two *Syzygium* species had lower photosynthetic rates than mature green leaves, whereas immature and mature green leaves from two additional

Syzygium species had similar photosynthetic rates. Young red *Corymbia gummifera* and *Cotyledon orbiculata* leaves also had lower photosynthetic rates than mature green leaves (Barker et al., 1997; Choinski et al., 2003). However, the lower photosynthetic rates observed in juvenile red leaves could have been due to the presence of anthocyanins or incomplete development of the photosynthetic apparatus (Turgeon, 1989).

Most studies evaluate photosynthetic rate between red and green leaves on a leaf area basis. For many gas exchange systems, leaf area is the default unit for reporting photosynthesis since the cuvette is of a known area. This is a good comparison if leaves are of similar thickness and chlorophyll content, but if leaf characteristics differ, photosynthesis per leaf area can be misleading. For example, Gould et al. (1995) reported red *Begonia pavonina* leaves had a higher A_{max} than green leaves (2.27 and 1.87 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$, respectively). Using their reported mean values for photosynthesis and chlorophyll, it can be calculated that red and green leaves have photosynthetic rates of 2.26 and 2.59 μmol $CO_2 \cdot g\ chl^{-1} \cdot s^{-1}$, respectively. Red *B. pavonina* leaves, therefore, had a higher photosynthetic rate per area, but green leaves had a higher photosynthetic rate than red leaves per unit chlorophyll (unknown if that difference would have been statistically significant).

Differences in leaf characteristics (leaf weight and chlorophyll content) were noted within each species (Table 3.3), so photosynthetic rates were compared on an area, fresh weight, dry weight, and chlorophyll basis. As noted in the results, differences in A_{max} per unit area for coleus were significant for cultivar but not leaf color (Table 3.2). On a weight basis, however, red coleus leaves had a higher A_{max} than green leaves. This

is because red leaves, on average, weighed less per unit area than green leaves (20.0 and 23.6 mg·cm^{-2} FW, 2.63 and 3.06 mg·cm^{-2} DW, respectively; Table 3.3). On a per unit chlorophyll basis, green leaves had a higher A_{max} than red leaves because red leaves tended to have more chlorophyll per unit area than the green leaves (red leaves averaged 6.4 µg chl·cm^{-2}, while green leaves averaged 4.4 µg chl·cm^{-2}; Table 3.3).

Differences in A_{max} in red and green switchgrass were almost the opposite of those observed in coleus. 'Heavy Metal' (green) had a higher A_{max} per unit area and per unit fresh weight than Ruby Ribbons^{TM} (red) (Table 3.2), but no significant differences were observed in A_{max} on a chlorophyll basis. 'Heavy Metal' has a more robust growth habit than Ruby Ribbons^{TM} when grown in the field or in containers (personal observation), which was reflected in its higher A_{max} per leaf area. 'Heavy Metal' also had three times more chlorophyll than Ruby Ribbons^{TM} per leaf area, which resulted in similar photosynthetic rates per unit chlorophyll.

Strikingly, the photosynthetic rate at a given irradiance relative to the photosynthetic rate at ambient irradiance (300 µmol·m^{-2}·s^{-1}; A_n=1) was almost identical for both switchgrass cultivars across the range of irradiances measured (Fig. 3.2F). At A_{max}, it was 1.70 for Ruby Ribbons^{TM} and 1.74 for 'Heavy Metal'. The photosynthetic rate at A_{max} relative to A_n at ambient irradiance varied between 1.18 and 2.39 in coleus (Fig. 3.2E), which waslikely due to differences in the convexity of the light response curve, as well as the irradiance at which light saturation occurred. Coleus cultivars with lower LSPs tended to have lower values for A_{max} (relative to A_n).

Red coleus leaves were thinner than green coleus leaves and based on visual observations, it appeared that red coleus leaves had a larger total leaf area than green leaves. Therefore, they may have had similar total mass per leaf (data not collected), but individual leaf discs from red leaves weighed less than those of identical size from green leaves. Additionally, red coleus tended to have more chlorophyll per unit area than green coleus, which contrasted with the similar chlorophyll contents observed for red and green coleus by Burger and Edwards (1996) but was similar to results observed in *Begonia pavonina* and *Triolena hirsuta* (Gould et al., 1995).

In coleus and switchgrass, Φ_{PSII} and qP decreased as irradiance increased, and ETR and NPQ increased as irradiance increased. Similar Φ_{PSII} values were observed between red and green leaves in both coleus and switchgrass. Likewise, in *Quercus coccifera*, red and green leaves had similar Φ_{PSII} when exposed to red light (Karageorgou and Manetas, 2006). Red *Q. coccifera* leaves, however, had higher Φ_{PSII} than green leaves when exposed to white light. Similarly, green and purple *Bauhinia variegata* pods had similar Φ_{PSII} when exposed to red light, but the purple pods had higher Φ_{PSII} under blue-green light (Smillie and Hetherington, 1999). It is possible that with our light quality (90% red and 10% blue) in the cuvette and the lack of absorption of red light by anthocyanins, some of the potential photoprotective effects provided by anthocyanins from absorption of some of the blue light was offset by a lack of red light absorption, resulting in no detectable differences in Φ_{PSII} between red and green leaves.

Photochemical quenching (qP) provides a relative measure of the oxidation state of the pool of Q_A, the electron acceptor for PSII (Maxwell and Johnson, 2000). When qP

= 1, all Q_A molecules are oxidized ("open") and able to accept electrons. When qP = 0, all Q_A molecules are reduced and unable to accept electrons. If Q_A molecules are unable to accept electrons, other molecules, including oxygen, can accept the electrons and form free radicals. Values of qP decreased from 1.0 at 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to around 0.2 at 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in coleus and switchgrass, and red and green leaves had similar rates of decline as irradiance increased. If anthocyanins have a photoprotective role, we would anticipate that qP would be higher in red leaves than in green leaves at similar incident irradiance levels. This is because anthocyanins would absorb some of the light and prevent it from reaching the underlying chloroplasts, resulting in reduced light capture by chloroplasts, fewer electrons needing to be transferred to an electron acceptor (like Q_A), and a higher qP. This, however, was not observed.

At high irradiance (2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$), coleus and switchgrass photosystems were only able to use 8-20% of the available light energy for photochemistry. The remainder of the absorbed light energy would have needed to be dissipated by other mechanisms. One mechanism, NPQ, increased in both species as irradiance increased. Red switchgrass leaves had higher NPQ than green leaves, but only at light intensities $\leq$ 700 $\mu mol \cdot m^{-2} \cdot s^{-1}$. However, NPQ was similar in red and green coleus leaves.

In both coleus and switchgrass, A_{max} was achieved at irradiances less than or equal to 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Likewise, at 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$, maximum rates of ETR and NPQ had been attained; chloroplasts were light-saturated and the capacity for electron flow from PSII to PSI and the capacity for non-photochemical quenching was maximized. Above 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$, values of Φ_{PSII} and qP were still declining,

however. Even though some of the excited electrons were still able to be accepted by available Q_A, the light reaction component of photosynthesis was, in essence, light-saturated. Therefore, at irradiances greater than 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$, photodamaging processes would have begun to occur, but since the leaves were exposed to high intensity irradiance for only a short duration, it was not long enough to observe a depression in photosynthesis.

The fact that anthocyanins did not provide any photoprotective role in this study when leaves were exposed to short-term fluctuations in irradiance concurs with results observed in other coleus cultivars (Burger and Edwards, 1996) and in young *Quercus coccifera* and *Syzygium* leaves (Karageorgou and Manetas, 2006; Woodall et al., 1998). While anthocyanins did not provide photoprotection against visible light in those studies, they were, however, able to provide protection in other ways, for example, against UV light in coleus (Burger and Edwards, 1996) and *Syzygium* (Woodall et al., 1998) and against herbivory in *Q. coccifera* (Karageorgou and Manetas, 2002).

Leaf color did not affect the intercellular CO_2 compensation point (C_i) or the carboxylation efficiency in coleus and switchgrass. C_i was lower in switchgrass than in coleus and carboxylation efficiency was higher in green switchgrass relative to red switchgrass or coleus. Switchgrass is a C_4, warm-season grass with the ability to concentrate CO_2 in bundle sheath cells, the site of carbon fixation (Osmond et al., 1982). Because of this unique mechanism and leaf anatomy, CO_2 does not compete as much with O_2 at the binding site on Rubisco (Lambers et al., 2008). As a result, rates of photorespiration are lower and net photosynthesis is positive at lower intercellular and

external CO_2 concentrations. CO_2 compensation points are typically between 0 and 5 $\mu mol \cdot mol^{-1}$ C_i in C_4 plants and between 40 and 50 $\mu mol \cdot mol^{-1}$ C_i in C_3 plants (Lambers et al., 2008), which was true of the two species in this study. Switchgrass C_i compensation points were approximately 0 $\mu mol \cdot mol^{-1}$ and coleus C_i compensation points ranged from 23-60 $\mu mol \cdot mol^{-1}$ (Table 3.4). At ambient external CO_2 (400 $\mu mol \cdot mol^{-1}$), average C_i was higher in coleus (262 $\mu mol \cdot mol^{-1}$) than in switchgrass (183 $\mu mol \cdot mol^{-1}$).

Maximum photosynthetic rates occurred at external CO_2 concentrations between 900 $\mu mol \cdot mol^{-1}$ and 1200 $\mu mol \cdot mol^{-1}$ in both species, regardless of leaf color. This range is similar to results reported for many greenhouse crops. Maximum photosynthetic rates occur at approximately 900 $\mu mol \cdot mol^{-1}$ for many potted plant species (Bierhuzen, 1983; Mortensen, 2000; Mortensen and Moe, 1983), and in another study, we observed A_{max} occurred between 400 and 800 $\mu mol \cdot mol^{-1}$ in 13 herbaceous ornamental species (Boldt et al., 2011b).

The photosynthetic rate at A_{max} in switchgrass was 1.7 to 1.9-fold higher than at ambient CO_2 (400 $\mu mol \cdot mol^{-1}$), and A_{max} in coleus was 1.7 to 2.2-fold higher than at ambient CO_2. Typically, CO_2 enrichment is more beneficial to C_3 than C_4 species (Ehleringer and Bjorkman, 1977), but increased rates of photosynthesis were comparable between coleus and switchgrass under short-term CO_2 supplementation. While Kimball (1983) observed, on average, a 28% increase in "yield" (dry weight, flower number, photosynthetic rate, etc.) in agricultural and horticultural crops when CO_2 was doubled, photosynthesis in switchgrass increased 50% and coleus increased 50-90% when CO_2 was doubled (from 400 $\mu mol \cdot mol^{-1}$ to 800 $\mu mol \cdot mol^{-1}$). It is unknown if, and by how

much, a depression in photosynthetic rate would occur after longer-term exposure to elevated CO_2 concentrations, but Mortensen (2000) noted a decline in photosynthesis in chrysanthemum and Rieger begonia after 3 weeks.

At each CO_2 concentration evaluated, leaf color in coleus did not affect photosynthetic rate when expressed on an area (Fig. 3.4E), weight (data not shown), or chlorophyll basis (Figure 3.4G). Green switchgrass benefited more from supplemental CO_2 than red switchgrass. On a leaf area basis, green switchgrass leaves had higher photosynthetic rates than red leaves at CO_2 concentrations of 600 $\mu mol \cdot mol^{-1}$ and higher. While A_{max} was lower in switchgrass than coleus, photosynthesis was still positive at very low CO_2 concentrations (lowest C_a measured was 50 $\mu mol \cdot mol^{-1}$).

At ambient CO_2 (and 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PAR* and 20 °C leaf temperature), leaves were not exposed to adverse environmental conditions. As CO_2 became limiting, the amount of light intercepted by light harvesting complexes may have become greater than that needed for photochemistry, resulting in photoinhibition (Hall and Rao, 1999). If this occurred, the presence of anthocyanins as a light screen could have been advantageous in order to limit the amount of light intercepted by light harvesting complexes (Close and Beadle, 2003). In coleus and switchgrass, however, no differences in Φ_{PSII}, ETR, or qP were observed between red and green leaves at ambient, supplemental, or CO_2-limiting concentrations. The presence of anthocyanins in leaves did not provide any photoprotection at CO_2-limiting conditions, but they also did not hinder photosynthetic efficiency at above-ambient CO_2 concentrations. The only difference was in leaf response to NPQ. In both species, NPQ decreased slightly as CO_2 increased. Green

coleus leaves had a higher NPQ than red leaves, whereas red switchgrass leaves had a higher NPQ than green leaves. It is possible that, had plants been exposed to below-ambient CO_2 concentrations for a longer duration (> 3 min), differences between leaf coloration may have emerged, but with short-term fluctuations in CO_2, neither leaf color provided a selective advantage.

Both suboptimal and supraoptimal temperatures can reduce net photosynthetic rate (Lambers et al., 2008; Savitch et al., 2001; Taylor and Rowley, 1971). Respiration and photorespiration increase as temperature increases, so even though gross photosynthetic rate may still increase with temperature, it is offset by increasing carbon losses due to photorespiration (Taiz and Zeiger, 2002). At low temperatures, reaction rates slow and light can become damaging at lower intensities (Demmig-Adams and Adams, 1992; Lambers et al., 2008). In our study, photosynthetic rates increased in both red and green switchgrass leaves as temperature increased from 10 °C to 30 °C (Fig. 3.6A). In coleus though, photosynthesis in red leaves increased as temperature increased, but photosynthesis in green leaves increased from 10 °C to 25 °C only, then decreased when temperatures increased from 25 °C to 30 °C. Photosynthetic rates between red and green leaves at 10 °C and 30 °C were similar, but green leaves had higher photosynthetic rates at intermediate temperatures (15 °C to 25 °C). Irradiance in our experiment was 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$. At higher irradiances, perhaps a greater difference between leaves may have occurred at low temperatures. For example, Pietrini et al. (2002) noted that red *Zea mays* leaves had similar photosynthetic rates as green leaves following a cold temperature, mild light stress (5 °C and 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 2 h) but higher

photosynthetic rates than green leaves following a cold temperature, high light stress (5 °C and 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 2 h). In addition, differences may have appeared if photosynthetic rates at lower temperatures (<10 °C) had been examined.

Anthocyanins in coleus leaves provided protection to PSII from low temperature, while in switchgrass leaves they did not. The maximum quantum yield (F_v/F_m) is typically around 0.83 in healthy, unstressed leaves, although it can vary with species, and F_v/F_m tends to decrease as plants are exposed to stress (Maxwell and Johnson, 2000). F_v/F_m decreased as temperature decreased below 20 °C in coleus and switchgrass. Red and green switchgrass leaves had similar decreases in F_v/F_m , whereas green coleus leaves had a much greater decrease in F_v/F_m than red leaves as temperature decreased from 20 °C to 10 °C. Smillie and Hetherington (1999) also noted a greater decrease in F_v/F_m in green *Bauhinia* pods than in purple pods following exposure to cool temperatures and high light. Janda et al. (1996) observed a decrease in F_v/F_m in *Zea mays* following a cold temperature stress, but only if the recovery occurred in the light rather than dark, indicating low temperature with high irradiance is especially damaging.

While anthocyanins in coleus were able to minimize the effects of low temperature on F_v/F_m, Φ_{PSII} and ETR were similar for red and green leaves at each temperature. In green leaves, protective mechanisms beside anthocyanins may help alleviate the effects of chilling in light-adapted coleus leaves. Rates of NPQ were similar for red and green coleus leaves at 10 °C and 15 °C, indicating there were no differences in the xanthophyll capacity, so other factors that we did not measure (i.e. antioxidants, other flavonoids) could have been upregulated more at low temperatures in green relative

to red leaves. Anthocyanins did not help switchgrass leaves minimize chilling injury at cool temperatures. In fact, Φ_{PSII} and ETR were lower and NPQ was higher in red switchgrass leaves relative to green leaves at comparable temperatures.

Overall, anthocyanins in coleus leaves provided protection against deleterious effects of low temperatures. Red leaves had less of a decline in F_v/F_m and photosynthesis than green leaves as temperatures decreased from 20 °C to 10 °C. They did not provide any photosynthetic advantage in coleus over the range of CO_2 concentrations (red and green leaves had similar rates of A_{max}, carboxylation efficiency, Φ_{PSII}, ETR, and qP), but may have provided a slight photoprotective role against high irradiance at ambient temperatures (A_{max} higher in red leaves per unit FW but similar to green leaves per unit area and similar rates of Φ_{PSII}, ETR, and qP). Anthocyanins in switchgrass did not provide a strong photoprotective role at high irradiance, cold temperatures, or CO_2-limiting conditions. Similar rates of Φ_{PSII}, ETR, and qP were observed in red and green leaves across the range of light intensities, temperatures, and CO_2 concentrations measured. We did measure their responses under a mix of red and blue light, so a stronger response may potentially be observed under white light (Burger and Edwards, 1996; Karageorgou and Manetas, 2006) or if we had exposed plants to temperature, irradiance, or CO_2 fluctuations for longer durations.

Table 3.1. Photosynthetic light response (A-I) curve parameters for coleus (*Solenostemon scutellarioides*) and switchgrass (*Panicum virgatum*) cultivars differing in leaf color. Individual response curves were developed for each plant, and values shown are the mean ± $_{SE}$ (n=5) for each cultivar. ANOVA *P*-values for each species are presented below the data.

Species	Cultivar	Leaf color	LCP[z] ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	LSP ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	AQE (ϕ)	Convexity (Θ)	R_d ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Amax ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$)
Coleus	'Big Red Judy'	Red	24 ± 7 a[y]	214 ± 23 cd	0.04 ± 0.01 ab	0.78 ± 0.09 ab	-0.9 ± 0.2 a	8.0 ± 1.2 bc
	'Twist and Twirl' red	Red	11 ± 4 a	166 ± 16 d	0.06 ± 0.01 ab	0.84 ± 0.07 ab	-0.8 ± 0.5 a	7.9 ± 1.0 bc
	'Versa Burgundy to Green'	Red	28 ± 9 a	185 ± 22 d	0.06 ± 0.01 a	0.86 ± 0.05 a	-1.6 ± 0.4 a	9.2 ± 0.4 abc
	'Versa Crimson Gold'	Red	21 ± 3 a	361 ± 49 ab	0.04 ± 0.01 ab	0.66 ± 0.12 ab	-0.8 ± 0.1 a	12.6 ± 0.9 a
	'Royal Glissade'	Red/green	18 ± 5 a	254 ± 30 bcd	0.03 ± 0.00 b	0.73 ± 0.12 ab	-0.4 ± 0.2 a	6.3 ± 0.4 c
	'Sedona'	Red/orange	31 ± 5 a	425 ± 37 a	0.02 ± 0.00 b	0.43 ± 0.09 b	-0.7 ± 0.1 a	9.0 ± 0.5 bc
	'LifeLime'	Green	24 ± 6 a	284 ± 30 bcd	0.03 ± 0.00 ab	0.59 ± 0.10 ab	-0.7 ± 0.2 a	7.4 ± 0.6 bc
	'Twist and Twirl' green	Green	14 ± 3 a	183 ± 14 d	0.05 ± 0.01 ab	0.90 ± 0.06 a	-0.8 ± 0.2 a	8.4 ± 0.2 bc
	'Versa Lime'	Green	28 ± 5 a	321 ± 40 abc	0.04 ± 0.01 ab	0.63 ± 0.12 ab	-0.9 ± 0.3 a	10.0 ± 1.3 ab
Switchgrass	Ruby Ribbons™	Red	17 ± 5 a	196 ± 24 a	0.03 ± 0.00 b	0.18 ± 0.16 a	-0.5 ± 0.2 a	5.6 ± 0.9 b
	'Heavy Metal'	Green	26 ± 7 a	237 ± 26 a	0.06 ± 0.00 a	0.47 ± 0.12 a	-1.5 ± 0.4 b	12.4 ± 1.9 a
ANOVA:								
Coleus	block		0.06 $_{NS}$[x]	0.02 *	0.91 $_{NS}$	0.12 $_{NS}$	0.19 $_{NS}$	0.12 $_{NS}$
	color		0.46 $_{NS}$	0.65 $_{NS}$	0.73 $_{NS}$	0.85 $_{NS}$	0.56 $_{NS}$	0.67 $_{NS}$
	cv (color)		0.16 $_{NS}$	<0.0001 ***	0.003 **	0.001 ***	0.23 $_{NS}$	<0.0001 ***
Switchgrass	block		0.03 *	0.49 $_{NS}$	0.82 $_{NS}$	0.09 $_{NS}$	0.07 $_{NS}$	0.80 $_{NS}$
	color		0.09 $_{NS}$	0.30 $_{NS}$	0.01 *	0.07 $_{NS}$	0.01 *	0.05 *

[z] light compensation point (LCP), light saturation point (LSP), apparent quantum efficiency (AQE, ϕ), convexity (Θ), respiration (R_d), maximum net photosynthesis (A_{max}, $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$)

[y] For each species, means within each column followed by a different letter are significantly different at $P<0.05$ using Tukey's HSD.

[x] NS,*,**,*** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively

Table 3.2. Light-saturated rates of maximum net photosynthesis (A_{max}) for coleus (*Solenostemon scutellarioides*) and switchgrass (*Panicum virgatum*) cultivars differing in leaf color. Photosynthetic rates (mean ± $_{SE}$, n=5) are presented on a leaf area (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), fresh weight (μmol $CO_2 \cdot g^{-1}$ FW$\cdot s^{-1}$), dry weight (μmol $CO_2 \cdot g^{-1}$ DW$\cdot s^{-1}$), and total chlorophyll basis (μmol $CO_2 \cdot mg^{-1}$ chl$\cdot s^{-1}$), as well as relative to ambient irradiance (300 μmol$\cdot m^{-2} \cdot s^{-1}$ *PAR*). Means within each column and cultivar followed by a different letter are significantly different at $P<0.05$ using Tukey's HSD.

Species	Cultivar	Leaf color	A_{max} (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)	A_{max} (μmol $CO_2 \cdot g^{-1}$ FW$\cdot s^{-1}$)	A_{max} (μmol $CO_2 \cdot g^{-1}$ DW$\cdot s^{-1}$)	A_{max} (μmol $CO_2 \cdot mg^{-1}$ chl$\cdot s^{-1}$)	A_{max} (relative to ambient irradiance)
Coleus	'Big Red Judy'	red	8.0 ± 1.2 bc	4.4 ± 0.6 ab	36.0 ± 5.3 a	14.5 ± 2.1 cd	1.38 ± 0.09 bc
	'Twist and Twirl' red	red	7.9 ± 1.0 bc	4.3 ± 0.5 ab	26.9 ± 3.3 ab	14.2 ± 1.8 d	1.21 ± 0.09 c
	'Versa Burgundy to Green'	red	9.2 ± 0.4 abc	4.0 ± 0.2 ab	33.0 ± 1.3 ab	5.8 ± 0.2 d	1.33 ± 0.06 bc
	'Versa Crimson Gold'	red	12.6 ± 0.9 a	5.4 ± 0.4 a	38,7 ± 2.9 a	14.1 ± 1.0 d	1.77 ± 0.15 b
	'Royal Glissade'	red/green	6.3 ± 0.4 c	3.9 ± 0.3 ab	30.7 ± 2.0 ab	31.3 ± 2.2 b	1.47 ± 0.13 bc
	'Sedona'	red/orange	9.0 ± 0.4 bc	4.3 ± 0.2 ab	36.8 ± 1.9 a	59.6 ± 3.6 a	2.39 ± 0.15 a
	'LifeLime'	green	7.4 ± 0.6 bc	2.8 ± 0.2 b	22.0 ± 1.8 b	23.8 ± 2.0 bc	1.67 ± 0.06 bc
	'Twist and Twirl' green	green	8.4 ± 0.2 bc	4.0 ± 0.1 ab	29.8 ± 0.7 ab	12.5 ± 0.3 d	1.18 ± 0.04 c
	'Versa Lime'	green	10.0 ± 1.3 ab	4.2 ± 0.5 ab	33.5 ± 4.2 ab	28.7 ± 3.6 b	1.79 ± 0.16 b
	means:	red	8.8 ± 0.5 a	4.4 ± 0.2 a	33.7 ± 1.4 a	16.0 ± 2.4 b	1.59 ± 0.09 a
		green	8.6 ± 0.5 a	3.7 ± 0.2 b	28.4 ± 1.9 b	21.7 ± 2.2 a	1.55 ± 0.09 a
Switchgrass	Ruby Ribbons™	red	5.6 ± 0.9 b	3.0 ± 0.5 b	6.6 ± 1.1 b	3.7 ± 0.6 a	1.70 ± 0.09 a
	'Heavy Metal'	green	12.4 ± 1.9 a	7.6 ± 1.2 a	17.6 ± 2.7 a	2.7 ± 0.4 a	1.74 ± 0.07 a
ANOVA:							
Coleus	block		0.12 $_{NS}$	0.13 $_{NS}$	0.12 $_{NS}$	0.05 $_{NS}$	0.44 $_{NS}$
	color		0.67 $_{NS}$	0.001 ***	0.01 *	0.03 *	0.57 $_{NS}$
	cv (color)		<0.0001 ***	0.03 *	0.01 *	<0.0001 ***	<0.0001 ***
Switchgrass	block		0.80 $_{NS}$	0.77 $_{NS}$	0.76 $_{NS}$	0.84 $_{NS}$	0.77 $_{NS}$
	color		0.05 *	0.04 *	0.03 *	0.32 $_{NS}$	0.74 $_{NS}$

Table 3.3. Comparison of leaf pigment content (anthocyanin and chlorophyll; n = 5) and mass (fresh and dry; n = 100) of nine *Solenostemon scutellarioides* (coleus) and two *Panicum virgatum* (switchgrass) cultivars varying in leaf coloration.

Species	Cultivar	Leaf Color	Chlorophyll ($\mu g \cdot cm^{-2}$)	Anthocyanin ($\mu g \cdot cm^{-2}$)	Fresh weight ($mg \cdot cm^{-2}$)	Dry weight ($mg \cdot cm^{-2}$)	Specific leaf area ($cm^2 \cdot g^{-1}$)
Coleus	'Big Red Judy'	Red	5.5	74.8	18.2	2.21	45.2
	'Twist and Twirl' red	Red	5.6	75.1	18.6	2.96	33.7
	'Versa Burgundy to Green'	Red	15.1	40.1	23.0	2.79	35.8
	'Versa Crimson Gold'	Red	8.9	43.0	23.2	3.25	30.8
	'Royal Glissade'	Red/green speckled	2.0	54.8	16.1	2.07	48.3
	'Sedona'	Orange-red	1.3	34.0	20.7	2.46	40.6
	'LifeLime'	Light green	3.1	1.5	26.1	3.36	29.8
	'Twist and Twirl' green	Dark green	6.7	3.7	20.8	2.82	35.4
	'Versa Lime'	Green	3.5	1.7	24.0	3.00	33.3
	means:	Red	6.4	53.6	20.0	2.63	39.1
		Green	4.4	2.3	23.6	3.06	32.6
Switchgrass	Ruby Ribbons™	Red	15.1	39.2	18.2	8.46	11.8
	'Heavy Metal'	Green	46.2	9.1	16.2	7.07	14.1

Table 3.4. Photosynthetic CO_2 response curve parameters for coleus (*Solenostemon scutellarioides*) and switchgrass (*Panicum virgatum*) cultivars differing in leaf color. Individual response curves were developed for each plant, and values shown are the means ($\pm$ SE, n=5) for each cultivar. ANOVA *P*-values for each species are presented below the data.

Species	Cultivar	leaf color	C_i comp. point[z]	C_a comp. point	C_a sat. point	Carboxylation efficiency	Amax ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$)
Coleus	'Big Red Judy'	red	60 ± 10 a[y]	57 ± 14 a	778 ± 66 abc	0.06 ± 0.02 a	18.9 ± 1.2 abc
	'Twist and Twirl' red	red	30 ± 8 a	15 ± 9 a	840 ± 16 ab	0.04 ± 0.01 a	16.7 ± 1.6 abc
	'Versa Burgundy to Green'	red	30 ± 9 a	26 ± 9 a	670 ± 61 abc	0.06 ± 0.01 a	24.4 ± 3.9 a
	'Versa Crimson Gold'	red	29 ± 5 a	22 ± 4 a	630 ± 37 c	0.06 ± 0.01 a	21.4 ± 0.8 ab
	'Royal Glissade'	red/green	34 ± 14 a	40 ± 17 a	866 ± 50 a	0.05 ± 0.01 a	14.1 ± 0.7 bc
	'Sedona'	red/orange	43 ± 14 a	41 ± 12 a	688 ± 28 abc	0.04 ± 0.01 a	12.3 ± 0.5 c
	'LifeLime'	green	39 ± 5 a	28 ± 8 a	643 ± 55 bc	0.06 ± 0.01 a	17.2 ± 1.3 abc
	'Twist and Twirl' green	green	34 ± 8 a	34 ± 13 a	757 ± 25 abc	0.05 ± 0.01 a	24.5 ± 2.8 a
	'Versa Lime'	green	23 ± 10 a	31 ± 9 a	708 ± 56 abc	0.05 ± 0.01 a	23.1 ± 1.4 a
Switchgrass	Ruby Ribbons™	red	ca. 0 a	ca. 0 a	672 ± 134 a	0.06 ± 0.03 a	11.8 ± 5.4 a
	'Heavy Metal'	green	ca. 0 a	ca. 0 a	541 ± 96 a	0.10 ± 0.04 a	13.3 ± 0.7 a
ANOVA:							
Coleus	block		0.16 $_{NS}$[x]	0.33 $_{NS}$	0.05 $_{NS}$	0.01 *	0.19 $_{NS}$
	color		0.21 $_{NS}$	0.76 $_{NS}$	0.17 $_{NS}$	0.99 $_{NS}$	0.10 $_{NS}$
	cv (color)		0.18 $_{NS}$	0.22 $_{NS}$	0.003 **	0.13 $_{NS}$	0.0002 ***
Switchgrass	block		0.50 $_{NS}$	0.48 $_{NS}$	0.69 $_{NS}$	0.87 $_{NS}$	0.53 $_{NS}$
	color		0.37 $_{NS}$	0.63 $_{NS}$	0.47 $_{NS}$	0.68 $_{NS}$	0.95 $_{NS}$

[z] intercellular CO_2 (C_i) compensation point ($\mu mol \cdot mol^{-1}$), external CO_2 (C_a) compensation point ($\mu mol \cdot mol^{-1}$), external CO_2 (C_a) saturation point ($\mu mol \cdot mol^{-1}$), carboxylation efficiency (CE), and maximum net photosynthesis (A_{max}; $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$)

[y] For each species, means within each column followed by a different letter are significantly different at $P<0.05$ using Tukey's HSD.

[x] NS,*,**,*** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively

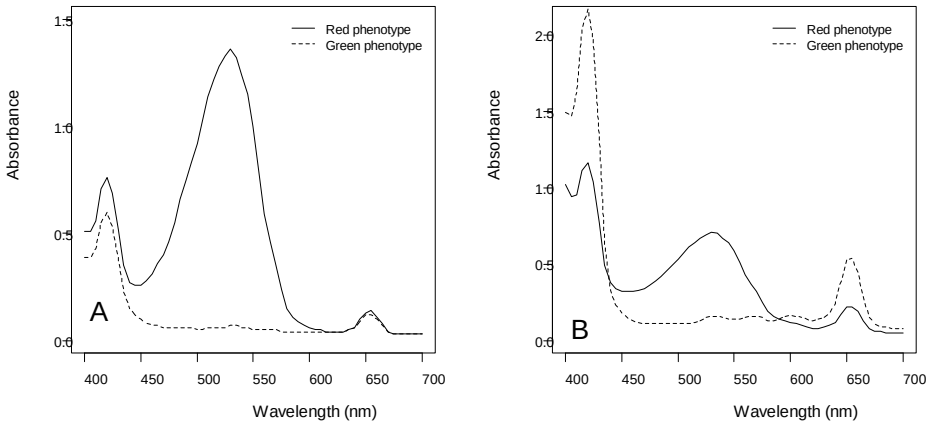

Fig. 3.1. Absorbance spectra of red and green leaved phenotypes in acidified methanol (99:1 methanol:HCl): A) *Solenostemon scutellarioides* (coleus) 'Twist and Twirl' red and green morphs and B) *Panicum virgatum* (switchgrass) 'Heavy Metal' (green) and Ruby Ribbons^{TM} (red). Maximum absorbance of the red-leaved phenotypes at 530 nm is due to the presence of anthocyanins. The smaller peak centered around 650 nm is due to chlorophyll absorbance.

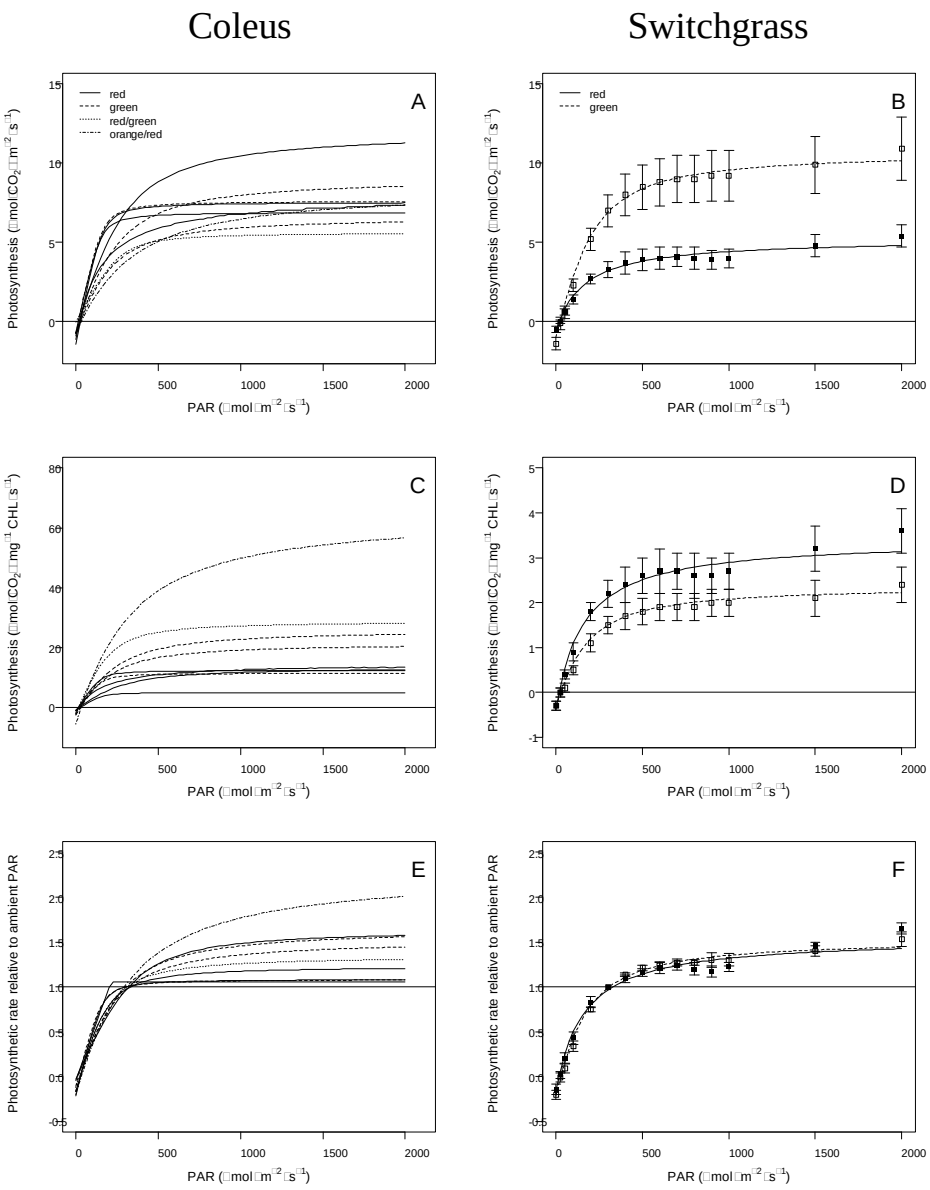

Fig. 3.2. Photosynthetic responses to irradiance (*PAR*, μmol·m^{-2}·s^{-1}) of coleus (A, C, and E) and switchgrass (B, D, and F) cultivars varying in leaf coloration. Net photosynthesis was calculated per leaf area (μmol CO_2·m^{-2}·s^{-1}; A and B) and total chlorophyll content (μmol CO_2·mg^{-1} chl·s^{-1}; C and D), as well as relative to ambient irradiance (300 μmol·m^{-2}·s^{-1} *PAR*; E and F): red leaves (—), green leaves (----), red/green leaves (·····), and red/orange leaves (-·-·-).

Coleus Switchgrass

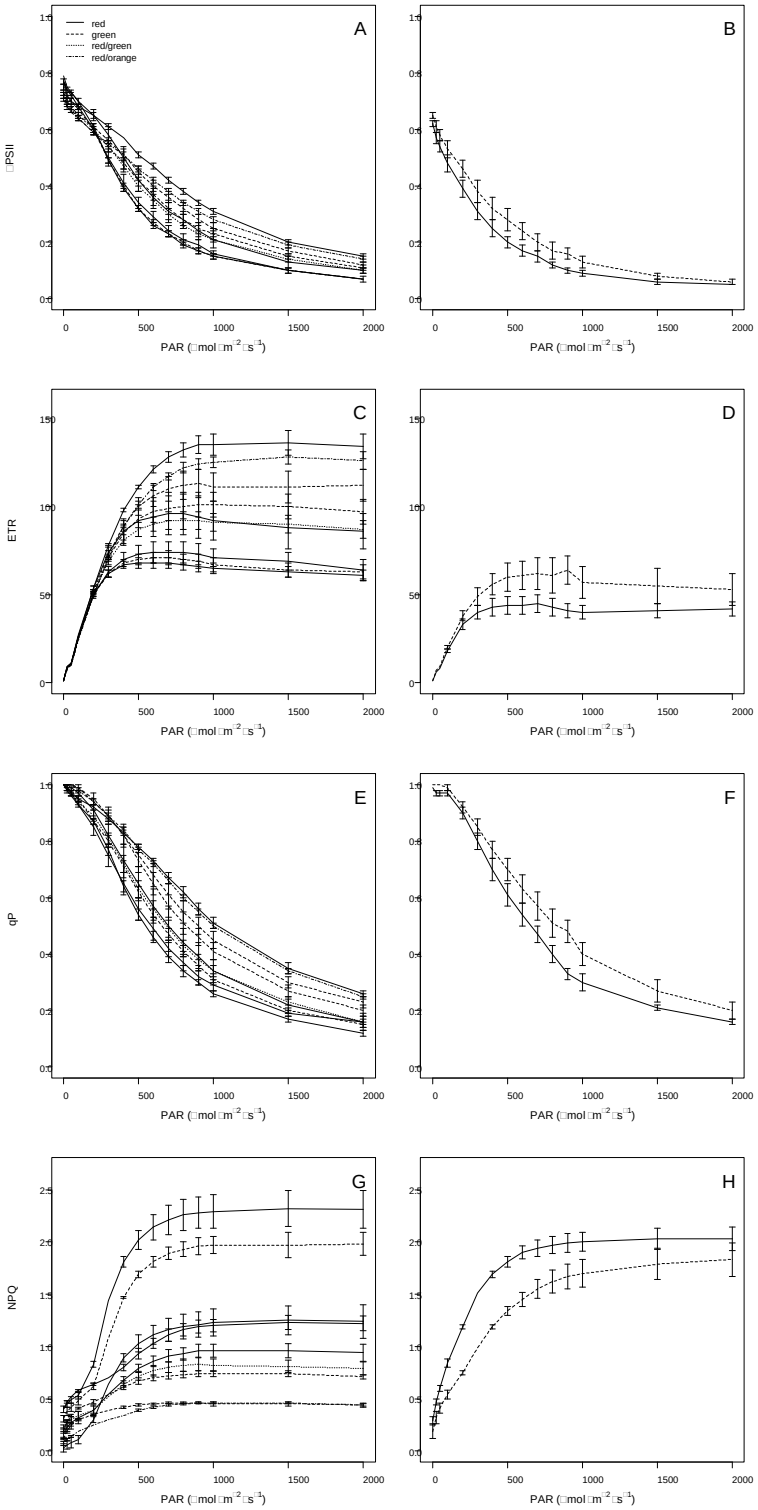

Fig. 3.3. Leaf fluorescence parameters of coleus (A, C, E, and G) and switchgrass (B, D, F, and H) cultivars varying in leaf coloration in response to irradiance (*PAR*, $\mu mol \cdot m^{-2} \cdot s^{-1}$): effective quantum yield, Φ_{PSII} (A and B), electron transport rate, ETR (C and D), photochemical quenching, qP (E and F), and non-photochemical quenching, NPQ (G and H). Plant leaf color denoted as follows: red leaves (—), green leaves (----), red/green leaves (·····), and red/orange leaves (-·-·-).

Coleus Switchgrass

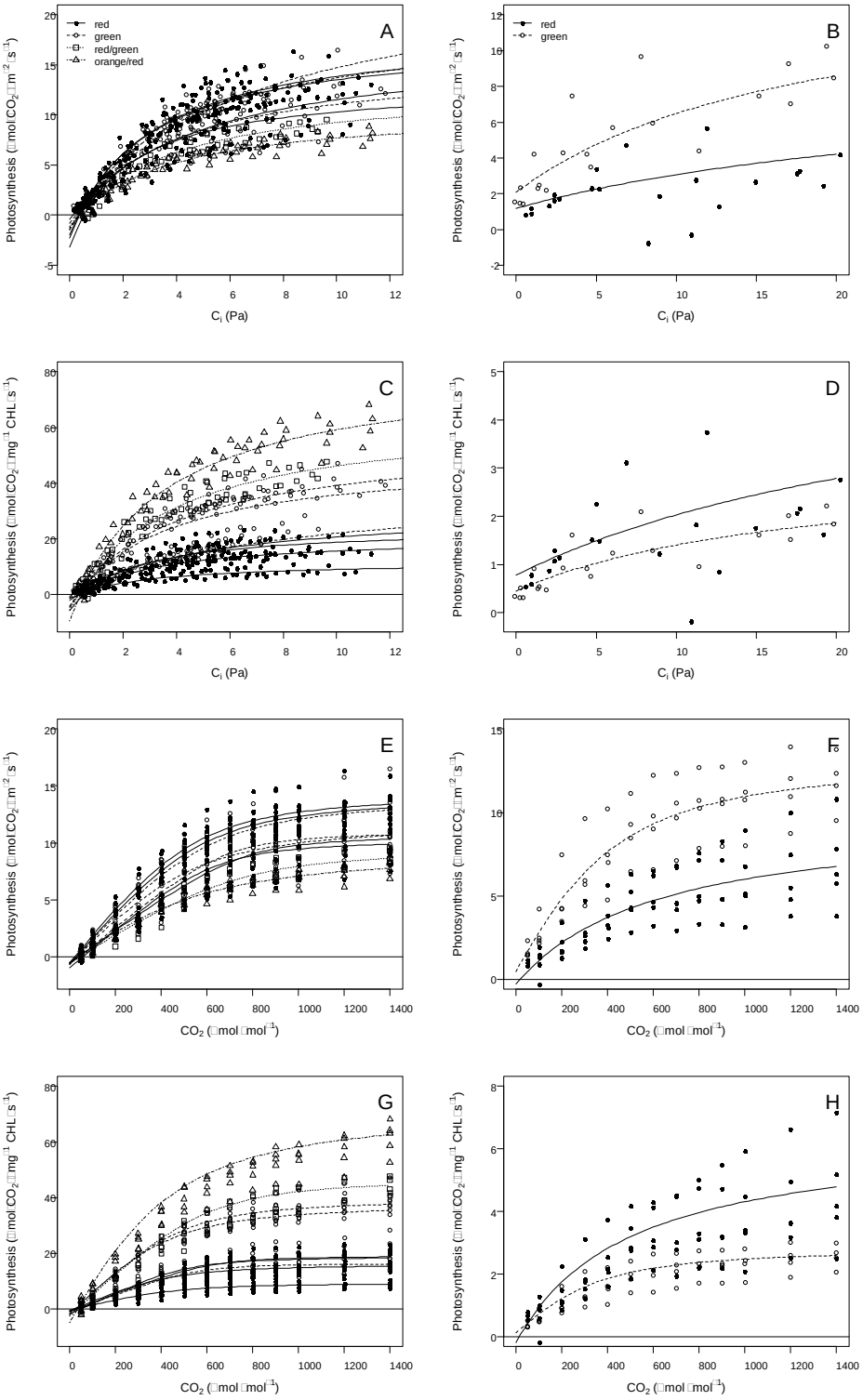

Fig. 3.4. Photosynthetic responses of coleus (A, C, E, and G) and switchgrass (B, D, F, and H) cultivars varying in leaf coloration to intercellular (C_i, Pa) and external (C_a, $\mu mol \cdot mol^{-1}$) CO_2 concentration. Net photosynthesis was calculated per leaf area ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$; A, B, E, and F) and total chlorophyll content ($\mu mol\ CO_2 \cdot mg^{-1}\ chl \cdot s^{-1}$; C, D, G, and H). The symbols represent the following: red leaves (●); green leaves (○); red/green leaves, coleus 'Royal Glissade' (□); and red/orange leaves, coleus 'Sedona' (Δ).

Coleus Switchgrass

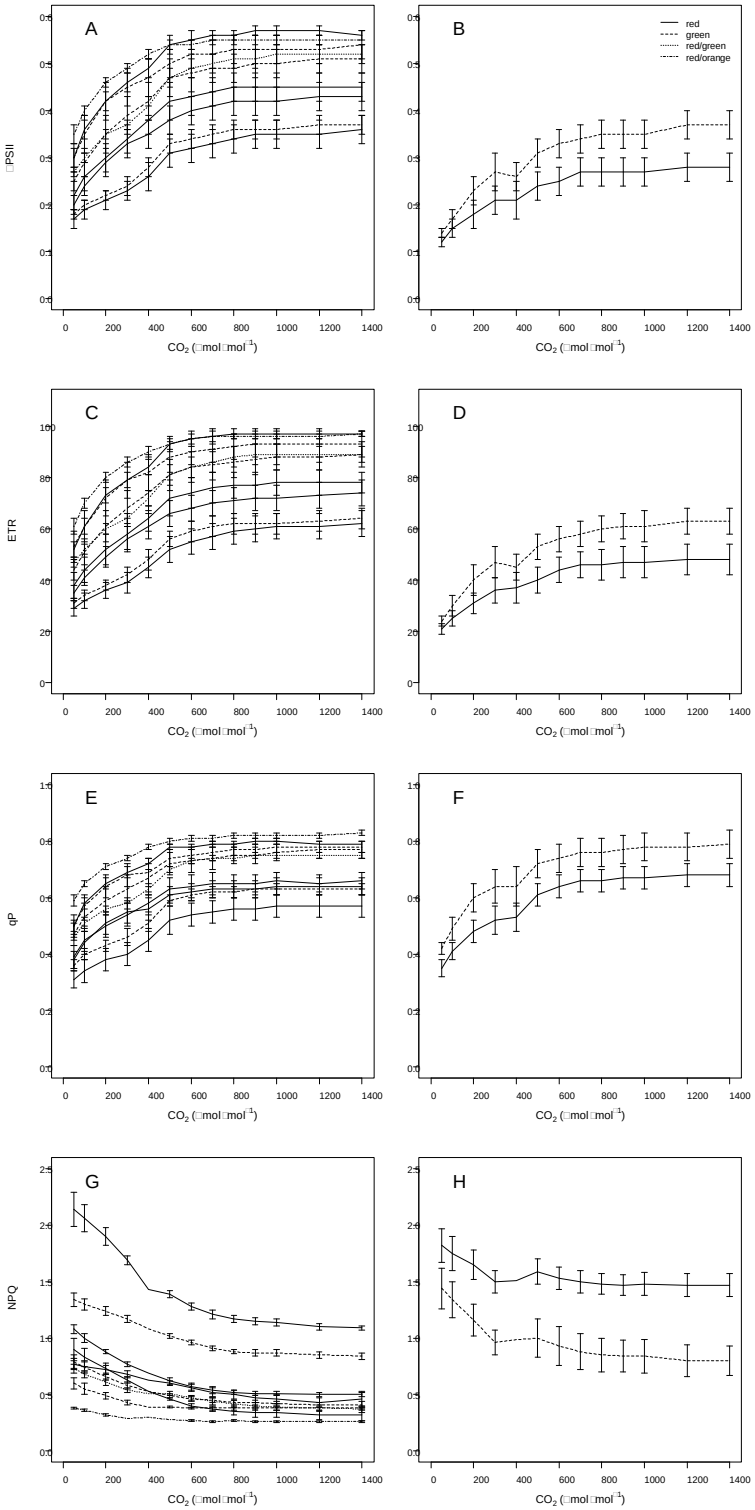

Fig. 3.5. Leaf fluorescence parameters of coleus (A, C, E, and G) and switchgrass (B, D, F, and H) cultivars varying in leaf coloration in response to external CO_2 concentration ($\mu mol \cdot mol^{-1}$): effective quantum yield, Φ_{PSII} (A and B), electron transport rate, ETR (C and D), photochemical quenching, qP (E and F), and non-photochemical quenching, NPQ (G and H). Plant leaf color denoted as follows: red leaves (—), green leaves (----), red/green leaves (·····), and red/orange leaves (-·-·-).

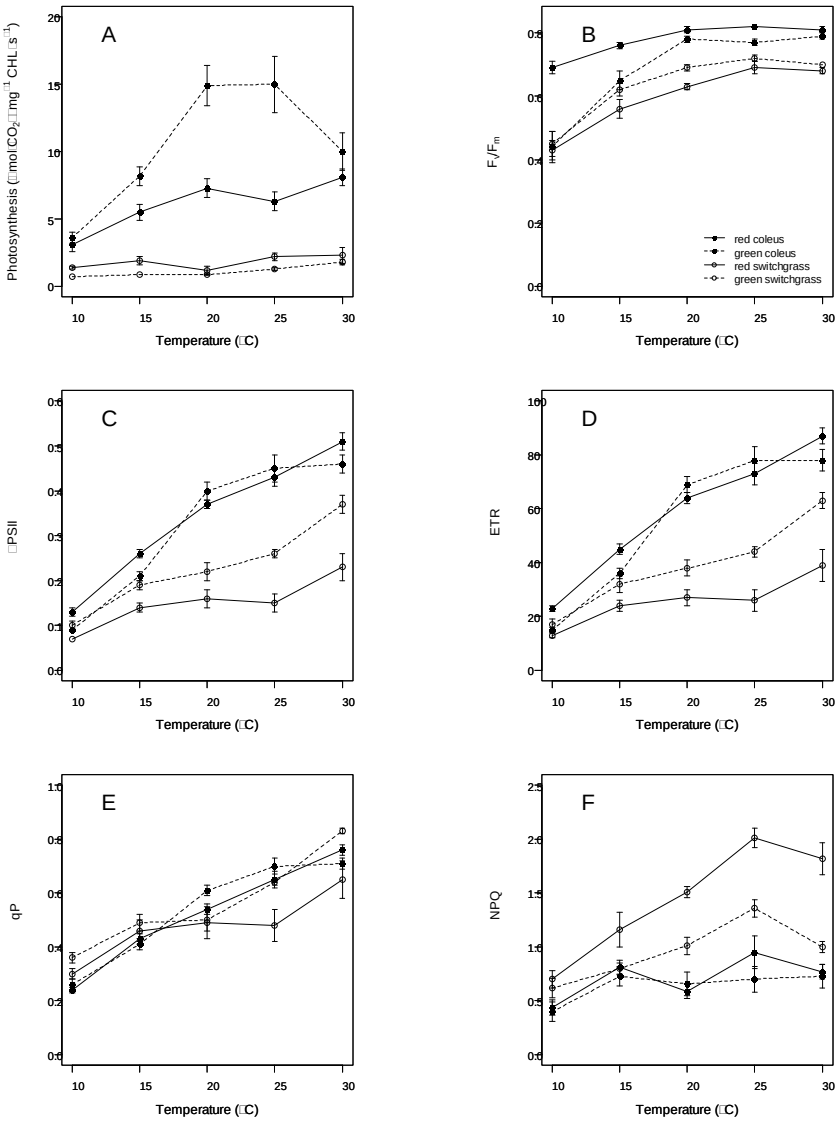

Fig. 3.6. Net photosynthesis (μmol $CO_2 \cdot mg^{-1}$ chl$\cdot s^{-1}$, A) and leaf fluorescence parameters in response to temperature (°C) in coleus and switchgrass cultivars varying in leaf coloration: B) maximum quantum yield, F_v/F_m, C) effective quantum yield, Φ_{PSII}, D) electron transport rate, ETR, E) photochemical quenching, qP, and F) non-photochemical quenching, NPQ. Symbols: red (—) or green leaves (----); coleus (○) or switchgrass (●).

Chapter 4

Leaf Coloration Affects the Severity of Photosynthetic and Chlorophyll Fluorescence Fluctuations During and After Light and Temperature Stress in *Solenostemon scutellarioides* and *Panicum virgatum*

Anthocyanins exhibited a strong photoprotective role in red *Solenostemon scutellarioides* L. (Codd) (coleus 'Big Red Judy') exposed to temperature and light stress, but not in *Panicum virgatum* L. 'RR1' Ruby Ribbons^{TM} (switchgrass). No protection against supplemental UV-B was found in either species following a brief exposure (<8 h). It appears that visible light attenuation may be a physiological function of anthocyanins in coleus. The low temperature/high irradiance (10 °C/600 µmol·m^{-2}·s^{-1}; LT/HL) treatment was the most stressful environment, and resulted in the lowest variable to maximum fluorescence ratios (F_v/F_m), net photosynthetic rates (P_n), effective quantum yields (Φ_{PSII}), photochemical quenching (qP), and electron transport rates (ETR), regardless of leaf color, after just 1 d exposure. F_v/F_m, P_n, and ETR continued to decrease while Φ_{PSII} and qP maintained constant, albeit very low, values as the exposure duration increased from 1 d to 4 d. The occurrence of anthocyanins reduced the severity of the LT/HL stress, especially in coleus. Red and green switchgrass had similar decreases in F_v/F_m after a 4 d exposure (-33% and -32%, respectively) and 4 d recovery (both -3%), but red coleus exhibited less of a decline in F_v/F_m than green coleus. F_v/F_m decreased only 19% in red coleus, compared to 78% for green coleus after a 4 d exposure. Red coleus also recovered faster (1 d) than green coleus (3 d) following return to ambient conditions (20

°C/300 μmol·m^{-2}·s^{-1}). Red coleus leaves had less of a decline in P_n (-58% red, -100% green), ETR (-45% red, -77% green), and Φ_{PSII} (-75% red, -89% green) after a 4 d exposure to LT/HL. Following a 4 d recovery, P_n had returned to pre-stress rates in red coleus but was still 43% lower in green coleus. Values of Φ_{PSII}, qP, and ETR were similar in red and green switchgrass after a 4 d LT/HL exposure and P_n was lower in red switchgrass, but the percent decline in all was similar or lower in red switchgrass than green switchgrass because the pre-stress values of these variables at ambient conditions were lower in red leaves. It appears that red switchgrass leaves are pre-disposed to have lower photosynthetic capacity and efficiency at ambient conditions, and the presence of anthocyanins helped equalize red leaf photosynthetic capacity and efficiency at photoinhibitory conditions, providing a minimal photoprotective role. Anthocyanin presence may be beneficial in the selection of cultivars of chilling-sensitive species that can better tolerate occasional exposure to cool temperatures and high light.

Introduction

Irradiance and temperature drive leaf photosynthetic rates and plant growth, but can also inhibit photosynthesis under extreme or stressful circumstances. Photoinhibition of photosynthesis occurs when more photons of light are absorbed by leaves than used in photochemistry (Krause, 1988). Photoinhibition can result from excessively high irradiance, reductions in carbon fixation, or damage to the thylakoid membrane (Krause, 1988). Photoinhibition results in a decrease in net photosynthetic rate (P_n), a decrease in

the ratio of variable to maximum chlorophyll fluorescence (F_v/F_m), and/or a decrease in photochemical efficiencies (Krause, 1988; Manetas, 2006; Steyn et al., 2002). Photoinhibition typically occurs under high irradiance in non-stressed, sun-adapted leaves and at lower irradiance in non-stressed, shade-adapted leaves or in stressed, sun- or shade-adapted leaves (Demmig-Adams and Adams, 1992).

Low temperature (typically 5 °C to 10 °C), especially in conjunction with high irradiance, can be especially damaging to photosystems (Krause, 1988). A combination of low temperature and high irradiance can increase the severity of 'stress' and delay recovery following removal of photoinhibitory conditions (Taylor and Rowley, 1971). At low temperatures, reduced rates of carbon fixation occur due to reduced enzyme activity, leading to an excess pool of reduced cofactors, a shortage of oxidized electron acceptors, and reduced rates of photon use. In combination with excess light capture at high irradiance, low temperature can quickly lead to the formation of an excess pool of excited electrons (Manetas, 2006).

Ultraviolet radiation can also damage leaves and reduce photosynthesis. UV-B (280-320 nm) can damage DNA, proteins, and membranes, resulting in lower light use efficiencies (Φ_{PSII}) or decreased photosynthetic rates (Chalker-Scott, 1999; Jansen et al., 1998). For example, in *Rhizophora apiculata* (mangrove), a simulated 10% ozone depletion resulted in a 45% decrease in P_n (Moorthy and Kathiresan, 1997). Teramura and Sullivan (1994) reported that photosynthesis of approximately one-third to one-half of species studied decreased as a result of excess UV-B exposure.

Photoinhibition resulting from excess UV-B, excess visible light, or low temperature, typically results from damage to photosystem II (PSII) reaction centers (Jansen et al., 1998; Krause, 1988; Stapleton, 1992; Teramura and Sullivan, 1994). Plants have developed a number of avoidance, tolerance, and repair mechanisms to effectively minimize photoinhibition, including changes in leaf orientation, leaf curling, formation of a thicker cuticle, formation of reflective hairs or salt crystals, accumulation of flavonoids, chloroplast movement, increased xanthophyll pool size, cyclic electron transport, increased photorespiration, increased Calvin Cycle enzyme pool size, oxygen radical scavenging by antioxidants, changes in the chlorophyll *a/b* ratio, and continual protein repair of PSII cores (Demmig-Adams and Adams, 1992; Jansen et al., 1998; Krause, 1988; Lambers et al., 2008; Stapleton, 1992; Steyn et el., 2002; Vuleta et al., 2011).

Anthocyanins, flavonoid pigments responsible for red and burgundy foliage coloration in many plant species (Chalker-Scott, 1999; Tanaka et al., 2008), can also help limit photoinhibition (Neill and Gould, 2003; Quina, 2009; Steyn et al., 2002). Anthocyanins may delay the onset of photoinhibition by absorbing light before it reaches chloroplasts or mitigate the severity of photoinhibition by eliminating oxygen radicals before they cause damage to thylakoid membranes (Neill and Gould, 2003). The primary function of anthocyanins may differ with species and be related to localization within a leaf. For instance, anthocyanin presence in the adaxial epidermis or in sub-epidermal cells is more likely related to the attenuation of excess UV-B or visible light, while

presence in photosynthetic palisade or spongy mesophyll cells is more likely related to a function in free-radical scavenging (Gould and Quinn, 1999; Neill and Gould, 2003).

The basis for the benefits anthocyanins may confer with respect to photoprotection from UV-B are not clear. The presence of anthocyanins reduced dimer formation (an indication of DNA damage) in cell suspension cultures of *Centaurea cyanus* L. (Takahashi et al., 1991) and minimized reductions in quantum use efficiency in *Coleus blumei* (Burger and Edwards, 1996) following exposure to UV-B radiation.

In *Cornus stolonifera*, foliar anthocyanins functioned as a visible light screen and protected against high irradiance (Feild et al., 2001). Anthocyanins also reduced photoinhibition in *Bauhinia variegata* pods (Smillie and Hetherington, 1999), *Begonia semperflorens* leaves (Zhang et al., 2011), *Cornus stolonifera* stems (Gould et al., 2010), *Galax urceolata* leaves (Hughes et al., 2005), and *Zea mays* leaves (Pietrini et al., 2002) following exposure to high-intensity white light.

Accumulation of anthocyanins is induced by the same conditions that result in photoinhibition (Manetas, 2006). Anthocyanins can accumulate in response to high irradiance (Beckwith et al., 2004; Boo et al., 1997; Hughes et al., 2005; Islam et al., 2005; Kleinhenz et al., 2003; Vuleta et al., 2011), low temperature, (Chalker-Scott, 1999; Hasdai et al., 2006; McKown et al., 1996; Pietrini and Massacci, 1998; Tignor et al., 1997; Tokuhisa et al., 1997), and UV-B radiation (Alexieva et al., 2001; Boo et al., 1997; Lindoo and Caldwell, 1978; Krizek et al., 1998; Maekawa et al., 2001; Mendez et al., 1999; Oren-Shamir and Levi-Nissim, 1997a; Pintér et al., 2007; Singh et al., 1999; Tsormpatsidis et al., 2008).

The ability of anthocyanins to attenuate light could be an especially significant coping mechanism if they were able to effectively protect against photodamage without reducing photosynthetic capacity in red (anthocyanic) leaves relative to green (non-anthocyanic) leaves. Many species with red leaves have lower photosynthetic rates under non-stressed conditions (Barker et al., 1997; Gould et al., 2002; Tuohy and Choinski, 1990; Woodall et al., 1998; Zhang et al., 2011) but higher or similar photosynthetic rates under stressful conditions (Burger and Edwards, 1996; Gould et al., 1995; Marini, 1986; Pietrini et al., 2002). These conflicting observations may be related to differences in leaf anthocyanin concentration, anthocyanin distribution, or the environmental conditions at which P_n was measured.

Our research objectives here were to determine the impact of short-term temperature/irradiance (less than 1 week) and UV-B (hours) exposure on P_n and chlorophyll fluorescence in red and green leaves of *Solenostemon scutellarioides* L. (Codd) (coleus; C_3 plant) and *Panicum virgatum* L. (switchgrass; C_4 plant) and to observe if the severity of photoinhibition and recovery interval following a stress differed between red and green leaves of the same species. We hypothesized that (1) the presence of anthocyanins would reduce the severity of a stress event and allow leaves to recover more quickly to pre-stress values following transfer back to ambient growing conditions in both species, (2) the severity of photoinhibition would increase in red and green leaves as the exposure interval increased, and (3) anthocyanins would be more beneficial in coleus leaves since they have larger leaves, are adapted to a partial-sun sun environment, and are more sensitive to chilling injury than switchgrass.

Materials and Methods

Expt. 1: Temperature/irradiance

Cuttings of *Solenostemon scutellarioides* (coleus) 'Big Red Judy' and 'LifeLime' (Pleasant View Gardens, Loudon, NH) were harvested from stock plants maintained in 16.5 cm diameter pots (volume = 1.3 L) in a greenhouse (University of Minnesota, St. Paul, MN). Greenhouse air temperature set points for stock plants and cutting propagation were 22 °C day/18 °C night and ambient irradiance. Plants received night interruption lighting (2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from incandescent bulbs nightly, 2200-0200 $_{HR}$) to keep them vegetative. Cuttings were trimmed to two nodes, stuck in a 50-count cell tray (volume = 110 cm^3) filled with LC-8 soilless media (SunGro Horticulture, Bellvue, WA) and placed under intermittent mist (5 s every 15 min) for 7 d until root formation occurred. Plants were then moved into the greenhouse and transplanted 2 weeks later into 10 cm diameter square pots (volume = 815 cm^2) filled with LC-8 soilless media. Temperature and lighting conditions were the same as those for the stock plants. Plants were pruned to one node 10 d after transplant.

Panicum virgatum (switchgrass) 'Heavy Metal' and 'RR1' Ruby Ribbons™ (Emerald Coast Growers, Pensacola, FL) plants were separated into divisions containing five to seven young tillers (< 5 cm tall). Divisions were transplanted into 10 cm diameter square pots with SB500, a high-porosity soilless media (SunGro Horticulture, Bellvue,

WA). Plants were watered as needed and fertilized weekly with 14.3 mM N from a 20N-4.4P-16.6K fertilizer (Peter's 20-10-20; The Scott's Co., Marysville, OH).

Four weeks after rooting, coleus and switchgrass plants were moved to growth chambers to provide a consistent environment prior to and during environmental stress treatments. The experiment was conducted twice (Spring and Fall 2011). Mean greenhouse temperatures and daily light integrals (DLI, $mol \cdot m^{-2} \cdot d^{-1}$) for each experiment are presented in Table 4.1. The growth chamber (3.3 m^2; Environmental Growth Chambers, Chagrin Falls, OH) was maintained at 20 ± 1 °C leaf temperature, a 16 h photoperiod (1100-0300 $_{HR}$), 50% relative humidity, and 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ irradiance (photosynthetically active radiation, *PAR*) provided with fluorescent and incandescent lamps. Plants were watered as needed and fertilized weekly with 14.3 mM N from a 20N-4.4P-16.6K fertilizer (Peter's 20-10-20; The Scott's Co., Marysville, OH). Treatments began 7 d later, following acclimation to the growth chamber environment.

Three plants of each cultivar were moved from ambient growth chamber conditions (20 ± 1 °C leaf temperature/300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ irradiance) to four temperature/irradiance ('stress') combinations [low temperature/low light (LT/LL; 10 °C leaf temperature/150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ irradiance), low temperature/high light (LT/HL; 10 °C/600 $\mu mol \cdot m^{-2} \cdot s^{-1}$), high temperature/low light (HT/LL; 30 °C/150 $\mu mol \cdot m^{-2} \cdot s^{-1}$), and high temperature/high light (HT/HL; 30 °C/600 $\mu mol \cdot m^{-2} \cdot s^{-1}$)] for 1, 2, or 4 d (coleus) or for 1, 2, 4, or 7 d (switchgrass) (growth chambers = 1.4 m^2),. A fifth treatment remained continuously at ambient conditions (20°C/300 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Plants were transferred

back to ambient growth chamber conditions after the exposure duration for a 'recovery' period (equal in duration to the treatment exposure).

Photosynthetic measurements were collected daily during the exposure and recovery intervals using a portable photosynthesis system (LI6400XT; LI-COR Biosciences, Inc., Lincoln, NE). A leaf chamber fluorometer (6400-40; LI-COR Biosciences, Inc., Lincoln, NE) was used to simultaneously measure gas exchange and chlorophyll fluorescence (cuvette leaf area = 2 cm^2). Actinic irradiance was supplied by a combination of red and blue light-emitting diodes (LEDs), with peaks at 630 nm and 470 nm, respectively (LI-COR, 2008). Temperature and irradiance conditions in the leaf chamber matched growth chamber conditions. Reference CO_2 was maintained at 380 ppm (µmol $CO_2 \cdot mol^{-1}$ air), flow rate was 400 µmol$\cdot s^{-1}$, and relative humidity was ambient.

Data were collected on fully-expanded mature leaves only. In coleus, a leaf from the fourth node below the shoot apex was selected. In switchgrass, one leaf from a vegetative stem was selected and centered within the cuvette. Since switchgrass leaves did not fill the entire cuvette, photosynthetic rates were adjusted based on actual leaf area within the cuvette (ranged between 0.8 cm^2 and 1.2 cm^2). Leaves of similar size and location on each plant were selected so that they would be of similar age and photosynthetic capacity. Dark-adapted variable to maximum fluorescence (F_v/F_m) values were collected in the morning (between 0900-1100 $_{HR}$), and light-adapted chlorophyll fluorescence and photosynthesis measurements were collected starting 1 h after lamps turned on (from 1200-2000 $_{HR}$).

Photosynthetic irradiance response curves were collected at the end of the exposure duration in all treatments, after plants were returned to ambient temperature and irradiance, and again during the recovery duration. A single leaf from each plant was illuminated with 900 $\mu mol \cdot m^{-2} \cdot s^{-1}$ until photosynthetic rate stabilized, and then an irradiance response curve was determined from 900 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$, decreasing at increments of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Data were recorded once stability parameters were met, following a minimum wait time of 2 min (maximum wait time of 3 min). Stability parameters were defined for photosynthesis, intercellular CO_2 concentration (C_i), conductance, and fluorescence. Values were calculated for maximum light-adapted quantum yield (F_v'/F_m'), effective quantum yield (Φ_{PSII}), electron transport rate (ETR), photochemical quenching (qP), and non-photochemical quenching (qN) (Maxwell and Johnson, 2000).

The experimental design was a randomized complete block design. The experimental layout was a split plot, with the temperature/irradiance treatment as the main plot, cultivar as the sub-plot, and repeated measures (day) on the sub-plot since data were collected from the same leaf on each plant throughout the experiment duration. Each species (coleus and switchgrass) was analyzed separately; within species, each treatment duration (1, 2, 4, or 7 d) was also analyzed individually. Analysis of variance was conducted using SAS (PROC MIXED; SAS 9.3, Cary, NC). Mean separation was performed when sources of variation were significant at $P<0.05$ using Tukey's HSD ($\alpha=0.05$).

Expt. 2: UV-B exposure

Plant culture for coleus and switchgrass were the same as for Expt 1. Coleus cuttings were harvested 27 Feb. 2012 and panicum were divided 20 Mar. 2012, following the protocol in Expt. 1. Greenhouse temperature and irradiance data are presented in Table 4.1. Plants were moved into growth chambers for treatments on 20 Apr. 2012. Growth chamber (1.4 m^2; Environmental Growth Chambers, Chagrin Falls, OH) conditions were 20 ± 1 °C leaf temperature, a 16 h photoperiod (0600-2200 $_{HR}$), 50% relative humidity, and 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ irradiance (photosynthetically active radiation, *PAR*) provided by fluorescent and incandescent lamps. Plants were watered as needed and fertilized weekly with 14.3 mM N from a 20N-4.4P-16.6K fertilizer (Peter's 20-10-20; The Scott's Co., Marysville, OH). Experimental treatments began 7 d after plants were moved into growth chambers to allow for acclimatization.

Plants were moved from ambient conditions (containing approximately 3 $\mu mol \cdot m^{-2} \cdot s^{-1}$ UV-B emitted by fluorescent lamps, peak at 313 nm) to a UV-B enriched environment (16 $\mu mol \cdot m^{-2} \cdot s^{-1}$ UV-B, peak at 313 nm) for 0, 1, 2, 4, or 8 h before transfer back to ambient conditions. Leaves for photosynthesis and chlorophyll fluorescence data were selected as described in Expt. 1. After a 15 min dark-adaption period, provided using a dark adapting clip (9964-091; LI-COR Biosciences, Inc., Lincoln, NE), F_o and F_m were measured and F_v/F_m was calculated. Photosynthesis and chlorophyll fluorescence measurements were collected using the LI6400XT (as described in Expt. 1). Within the cuvette, leaf temperature was maintained at 22 °C ± 0.5 °C (mean ± $_{SD}$), reference CO_2 was 380 ppm ($\mu mol\ CO_2 \cdot mol^{-1}$ air), flow rate was 400 $\mu mol \cdot s^{-1}$, and relative humidity

was ambient (53% ± 8%). Leaves were illuminated with 600 µmol·m^{-2}·s^{-1} irradiance until leaf photosynthesis stabilized, then photosynthetic light response curve data were determined from 600 µmol·m^{-2}·s^{-1} to 0 µmol·m^{-2}·s^{-1}, decreasing at 100 µmol·m^{-2}·s^{-1} increments. F_v'/F_m', Φ_{PSII}, ETR, qP, and NPQ were calculated from chlorophyll fluorescence values.

The experimental design was a randomized complete block design, with two factors (UV duration and *PAR*). The two species, coleus (n=3) and switchgrass (n=4), were analyzed separately. Analysis of variance was conducted using SAS (PROC MIXED with a repeated statement, SAS 9.3; Cary, NC), with significance at $P<0.05$. Mean separation was performed for significant sources of variation using Tukey's HSD ($\alpha=0.05$).

Results

Expt. 1: Short-term fluctuations in temperature and irradiance

Data are presented for 1, 2 and 4 d exposure durations in coleus (7 d exposure for switchgrass plants only) because 'LifeLime' (green) coleus leaves in the low temperature/high irradiance (LT/HL; 10 °C/600 µmol·m^{-2}·s^{-1}) treatment had visible symptoms of chilling injury (epinasty, small necrotic spots along leaf margins, and leaf curl) by day 3 and leaves abscission on day 5, eliminating the possibility of complete data collection.

F_v/F_m

In both species, F_v/F_m was not affected by a 30 °C exposure at either irradiance but declined substantially when plants were exposed to 10 °C, especially in combination with high irradiance (600 µmol·m^{-2}·s^{-1}) (Fig. 4.1). Red coleus leaves exhibited less of a decline in F_v/F_m than green leaves following a low temperature (10 °C) exposure and recovered more quickly following a return to ambient conditions (20 °C, 600 µmol·m^{-2}·s^{-1}). During a 1, 2, or 4 d exposure to 10 °C, there was no change in F_v/F_m in red ('Big Red Judy') leaves exposed to low irradiance (LT/LL; 150 µmol·m^{-2}·s^{-1}), but a decrease was observed in green ('LifeLime') leaves. After a 4 d exposure to LT/LL, F_v/F_m decreased by 2% in red coleus leaves and 11% in green coleus leaves (Fig. 4.1A and B). The decrease in F_v/F_m was temporary, however, and leaves recovered to pre-treatment values following return to ambient conditions. Exposure to low temperature and high irradiance (LT/HL; 10 °C/600 µmol·m^{-2}·s^{-1}) resulted in a decrease in F_v/F_m in both red and green coleus, although red leaves were less affected than green leaves. 'Big Red Judy' declined from an initial F_v/F_m of 0.84 to 0.68 after 4 d in LT/HL (a 19% decrease), while 'LifeLime' decreased from 0.83 to 0.18 (a 78% decrease) (Fig. 4.1A and B). 'Big Red Judy' required only 1 d to recover to pre-treatment values of F_v/F_m, but 'LifeLime' required 3 d to recover (after 4 d recovery at ambient conditions, F_v/F_m was 2% lower than pre-treatment values in red leaves and 7% lower in green leaves).

Since exposure to 30 °C did not affect F_v/F_m in red or green coleus at low or high irradiance, we concluded that high irradiance was more detrimental to PSII in combination with low temperatures than with high temperatures. During every day of the

4 d exposure at 10 °C, F_v/F_m was higher in plants exposed to LL relative to those exposed to HL, whereas at 30 °C, F_v/F_m was similar in LL and HL-exposed plants. Likewise, low temperature was more detrimental in conjunction with high irradiance than with low irradiance. At 150 μmol·m^{-2}·s^{-1} (LL), plants exposed to 10 °C and 30 °C had similar rates of F_v/F_m, whereas at 600 μmol·m^{-2}·s^{-1}, F_v/F_m was greater at 30 °C than at 10 °C.

In switchgrass, however, red ('RR1' Ruby Ribbons™) and green ('Heavy Metal') cultivars exhibited similar decreases in F_v/F_m after a 4 d exposure to 10 °C (Fig. 4.1C and D). F_v/F_m significantly declined after only 1 d at 10 °C under both low (LL; 150 μmol·m^{-2}·s^{-1}) and high irradiance (HL; 600 μmol·m^{-2}·s^{-1}), although the decline was greater under high irradiance (from 0.74 to 0.69 in LT/LL and from 0.74 to 0.63 in LT/HL, pooled across leaf color). F_v/F_m continued to decline as exposure duration increased and after 4 d at 10 °C, F_v/F_m was 0.63 in LT/LL and 0.51 in LT/HL. After transfer back to ambient conditions (20 °C, 600 μmol·m^{-2}·s^{-1}), F_v/F_m gradually increased and leaves recovered to pre-treatment values after 4 d.

Following a 7 d exposure to 10 °C, red switchgrass exhibited a greater decline in F_v/F_m than green switchgrass at low and high irradiance. F_v/F_m declined by 17% in red leaves and 12% in green leaves exposed to LT/LL and by 67% in red and 52% in green switchgrass exposed to LT/HL (data not shown). F_v/F_m recovered in red and green leaves exposed to LT/LL but did not recover following exposure to LT/HL (red and green leaves were still 31% and 13% lower, respectively, than initial F_v/F_m 7 d after return to ambient conditions).

Net photosynthesis

Net photosynthesis (P_n) was measured at ambient chamber conditions. Therefore, during the exposure duration, P_n in each treatment was measured at each temperature/ irradiance combination, but during the recovery interval, P_n was determined at the same environmental conditions (20 °C/300 μmol·m^{-2}·s^{-1}) in all plants. In general, P_n was greater at 30 °C than at 10 °C and it was highest in the HT/HL treatment during the exposure duration (Fig. 4.2).

After a 4 d exposure, P_n in green switchgrass was 2.0 μmol CO_2·m^{-2}·s^{-1} in the LT/LL treatment and 1.8 μmol CO_2·m^{-2}·s^{-1} in the LT/HL treatment, 75% and 78% lower, respectively, than untreated plants maintained continuously in ambient conditions (Fig. 4.2C). P_n in red switchgrass was 0.5 and 1.3 μmol CO_2·m^{-2}·s^{-1} in the LT/LL and LT/HL treatments, respectively (Fig. 4.2D), which was 74% and 32% lower than P_n in untreated plants. Further reductions in P_n were observed if the exposure duration was increased to 7 d. P_n was 96% lower in green switchgrass and 100% lower red switchgrass after 7 d at LT/HL. After a 7 d recovery, red and green switchgrass leaves exposed to LT/LL had recovered, but P_n in red and green leaves exposed to LT/HL had not recovered and was still about 70% lower than P_n in unstressed plants.

While red leaves did not confer a photosynthetic advantage during a 10 °C exposure in switchgrass, the presence of anthocyanins resulted in a lower depression in P_n in red coleus leaves during a 10 °C exposure compared to green leaves. After a 4 d stress, P_n relative to unstressed plants was 87% and 100% lower in green coleus exposed

to LT/LL and LT/HL, respectively, while P_n in red coleus was 70% and 58% lower in leaves exposed to LT/LL and LL/HL, respectively (Fig. 4.2A and B).

Red coleus leaves exposed to low or high irradiance at 10 °C had similar P_n as unstressed plants after a 4 d recovery. In contrast, green leaves exposed to 10 °C and low light recovered within 4 d, but those exposed to 10 °C and high light still had not recovered completely after 4 d (P_n was 43% lower than unstressed plants).

At 10 °C, green leaves of both species had a greater depression in P_n at high light than low light, while red leaves had a greater depression in P_n at low light than high light. At low temperatures, green leaves appeared to utilize incoming light at low irradiance but not at high irradiance and photoinhibition ensued. The presence of anthocyanins in the upper epidermis can limit light capture by chloroplasts (Pietrini et al., 2002), and at 10 °C and low irradiance, this did not appear to be advantageous since they were intercepting some light energy that could have been used in photochemistry. However, at high irradiance, the screening function of anthocyanins may have prevented excess light energy from reaching underlying chloroplasts, resulting in a delay in or lower degree of photoinhibition and P_n was higher than at lower irradiances because more light energy iwas being used for photosynthesis.

Effective quantum yield (Φ_{PSII})

Effective quantum yield (Φ_{PSII}) was similar in red and green coleus (0.57 and 0.56, respectively) under ambient temperature and irradiance. Switchgrass leaves had lower Φ_{PSII} than coleus under ambient conditions and green switchgrass leaves (0.39) had

a higher Φ_{PSII} than red leaves (0.16). Since red switchgrass leaves had a lower 'unstressed' Φ_{PSII}, comparisons were made both for percent change from ambient and numerically.

Effective quantum yield should be highest at low irradiance and decrease as irradiance increases. Within each temperature, leaves exposed to 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (LL) had a higher Φ_{PSII} than leaves exposed to 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (HL) during the exposure duration, regardless of species or leaf color (Fig. 4.3). Effective quantum yield should also be greater at higher temperatures (up to some biological maximum) due to quicker rates of enzyme activity in electron transport and carbon fixation, and this was observed. At each irradiance, leaves exposed to 30 °C (HT) had a higher Φ_{PSII} than leaves exposed to 10 °C during the exposure interval.

In switchgrass and coleus, red and green leaves had similar patterns of Φ_{PSII} during the 4 d exposure and subsequent 4 d recovery period (Fig. 4.3). There was no significant interaction between cultivar, treatment, and exposure duration (Table 4.2). HT/LL had the greatest Φ_{PSII} during the 4 d exposure, approximately 0.50 in switchgrass and 0.75 in coleus (pooled across leaf color; Fig. 4.3). HT/HL and LT/LL had intermediate values of Φ_{PSII}. LT/HL had the lowest Φ_{PSII} throughout the 4 d exposure, ranging between 0.06 and 0.08 in switchgrass and 0.10 and 0.16 in coleus. Values remained relatively stable during the stress exposure (except for LT/LL, which declined incrementally from 1S to 4S in both species; Fig. 4.3). Following transfer back to ambient conditions, plants in all treatments had similar Φ_{PSII} within 2 d.

The LT/HL treatment exhibited the greatest decline in Φ_{PSII}. Green switchgrass leaves exhibited a greater reduction in Φ_{PSII} than red switchgrass; after a 4 d exposure, Φ_{PSII} was 63% lower in red leaves and 85% lower in green leaves relative to unstressed plants. Likewise, Φ_{PSII} was 75% lower in red coleus and 89% lower in green coleus relative to unstressed plants after a 4 d exposure to LT/HL. Red and green switchgrass and red coleus in the LT/HL treatment had Φ_{PSII} comparable to unstressed plants after a 4 d recovery at ambient conditions, but green coleus leaves were still 27% lower than unstressed plants.

Photochemical quenching (qP)

Values of qP in untreated switchgrass were 0.79 in green and 0.46 in red leaves, and unstressed coleus values were 0.79 in green and 0.80 in red leaves. Red switchgrass leaves were pre-disposed to have lower qP than green switchgrass at ambient conditions, while red and green coleus leaves had very similar values of qP. Trends in qP in response to changes in temperature and irradiance were very similarto those observed for Φ_{PSII}.

The highest values of qP were observed in the HT/LL treatment in both species during the exposure duration (greater than 0.90), and the lowest values were observed in the LT/HL treatment (Fig. 4.4). After 1 d of a 4 d exposure, qP in the LT/HL treatment was lower than that of the other temperature/irradiance treatments in both taxa (0.27 in switchgrass and 0.31 in coleus, pooled across cultivar). The decline in qP was not permanent, however, and values were similar to those of the other treatments within 1 d

of transfer back to ambient conditions. On a percent basis, both coleus cultivars and green switchgrass experienced approximately a 65% reduction after 4 d in LT/HL, while red switchgrass was less affected (43% decrease in qP). This is likely due to the lower inherent qP in red switchgrass under ambient, non-stressed conditions. The percent decrease in qP in switchgrass following a 7 d exposure in LT/HL was very similar to that observed in the 4 d exposure (66% and 40% decrease in green and red switchgrass, respectively).

Exposure to low temperature, but at a lower irradiance (LT/LL) resulted in a less severe decrease in qP after a 1 d exposure than in LT/HL. Unlike the relatively constant values of qP observed in LT/HL as the exposure interval progressed, qP in plants exposed to LT/LL continued to decrease as the exposure duration progressed, indicating a continued increase in photoinhibition. The resulting decrease in qP after 4 d was similar in red and green coleus (22% and 26% decrease, respectively) and green switchgrass (23% decrease), but was much lower in red switchgrass (2% decrease).

Non-photochemical quenching (qN)

Red and green leaves in both taxa responded in a similar manner to the imposed treatments. In the 4 d stress exposure, there was no significant interaction between cultivar, treatment, and duration (Table 4.2). An interaction between treatment and duration was significant, however, in both species. Exposure to LT/LL did not impact qN in either species (similar values were observed across the entire exposure and recovery interval) (Fig. 4.5). Exposure to HT/LL resulted in a lower qN than that

observed at ambient conditions in both species. Exposure to high irradiance, at high or low temperature (LT/HL and HT/HL), resulted in higher qN in switchgrass during the 4 d exposure than in the subsequent 4 d recovery at ambient conditions, but qN was similar during the stress and recovery intervals in coleus.

In both species, observed differences in qN at the end of the 4 d stress exposure were the result of treatments and independent of leaf color. Differences in qN were observed between high and low irradiance at 30 °C, but not 10 °C. At 30 °C, qN was greater in HL than in LL – in switchgrass, qN was 0.81 in HL and 0.43 in LL and in coleus, qN was 0.60 in HL and 0.14 in LL. Also, differences in qN were observed between high and low temperature at low, but not high, irradiance. At 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$, qN was greater in LT than in HT – in switchgrass, qN was 0.71 at 10 °C and 0.43 at 30 °C and in coleus, qN was 0.54 at 10 °C and 0.14 at 30 °C.

Electron transport rate (ETR)

Changes in electron transport rate were cultivar specific for switchgrass but were similar between red and green coleus in response to treatment and duration (Table 4.2). Within 1 d of transfer to treatments, in both species, plants exposed to HT/HL had higher ETRs than plants that remained at ambient conditions. Plants in the other three treatments had lower ETRs relative to plants in ambient conditions (Fig. 4.6). After a 4 d exposure, ETR was greatest in HT/HL and lowest in LT/LL and LT/HL. ETR decreased 68% in green and 58% in red switchgrass and 77% in green and 45% in red coleus after 4 d in LT/HL.

Red and green coleus had similar patterns of recovery after return to ambient conditions. Within 2 d, all treatments had a similar ETR as untreated plants (Fig. 4.6A and B). Differential responses were observed in red and green switchgrass. As with coleus, all green switchgrass treatments were similar to untreated plants within 2 d of transfer back to ambient conditions. In red switchgrass, after a 2 d recovery, ETR of HT/HL and LT/LL treatments were lower than the other treatments (and untreated plants). ETR continued to increase in plants exposed to LT/LL, and after 4 d all but HT/HL were similar to untreated plants.

Under ambient conditions, red and green coleus plants had similar rates of electron transport (66 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 65 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively), but red switchgrass leaves had lower rates of electron transport than green leaves (38 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively).

Irradiance response curves during stress exposure and recovery

Irradiance response curves from coleus and switchgrass leaves at the end of a 4 d exposure to 10 °C at low and high irradiance (LT/LL and LT/HL) had lower rates of P_n, Φ_{PSII}, and ETR relative to response curves measured prior to the start of the low temperature exposure. After transfer back to ambient conditions, plants, in general, recovered gradually to pre-treatment values.

Net photosynthesis after 4 d at 10 °C was lower than pre-treatment P_n at saturating irradiance (900 $\mu mol \cdot m^{-2} \cdot s^{-1}$) in red and green coleus leaves exposed to both LT/LL and LT/HL (Fig. 4.7), 75% and 98% lower in ‘LifeLime’ and 65% and 64% lower

in ‘Big Red Judy’, respectively. After 2 d recovery at ambient conditions, both red and green coleus leaves in the LT/LL treatment had recovered to pre-treatment rates of P_n (insignificant differences at α=0.05), whereas recovery was more gradual in red and green coleus leaves exposed to LT/HL. Red coleus leaves had a more complete recovery than green leaves. At the end of the recovery interval (4 d at ambient conditions), P_n at saturating irradiance was 16% and 38% lower than pre-treatment rates of P_n in green leaves exposed to LT/LL and LT/HL, respectively, whereas, P_n was 5% lower and 8% higher in red leaves exposed to LT/LL and LT/HL, respectively.

At all irradiances (from 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 900 $\mu mol \cdot m^{-2} \cdot s^{-1}$), Φ_{PSII} was lowest in red and green coleus leaves after 4 d at 10 °C (Fig. 4.8), with a greater decrease occurring in combination with high irradiance. At LT/LL, red and green coleus leaves had a similar decline in Φ_{PSII} after a 4 d exposure (64% and 68%, respectively, at saturating irradiance) and similar rates of recovery. At high irradiance, however, red leaves had less of a decline than green leaves (63% lower in red leaves, 87% lower in green leaves at saturating irradiance). Green coleus leaves in the LT/HL treatment also required a longer duration to recover to pre-treatment values of Φ_{PSII}. Red leaves recovered after 4 d at ambient conditions, but green leaves were still not fully recovered after 4 d at ambient conditions (43% lower than pre-stress Φ_{PSII}).

Electron transport rate was lower in both red and green coleus after a 4 d exposure at LT/LL or LT/HL. Red coleus leaves had a similar depression in ETR compared to pre-treatment rates in LT/LL and LT/HL at saturating irradiance (64% and 63%, respectively), while green coleus leaves exhibited a greater decline at high irradiance

(68% decrease in LT/LL and 87% decrease in LT/HL) (Fig. 4.9A and B). Red and green leaves in the LT/LL treatment recovered within 2 d of return to ambient conditions, but those in the LT/HL treatment required a longer interval to recover. Red leaves recovered after 4 d (4% lower than pre- treatment), but green leaves had not fully recovered after 4 d (still 47% lower) (Fig. 4.9C and D).

Expt. 2: Supplemental UV-B irradiation

At ambient irradiance (150 $\mu mol \cdot m^{-2} \cdot s^{-1}$), red and green switchgrass had similar responses across all UV-B durations (no interaction between cultivar and duration; Table 4.3) for P_n and all chlorophyll fluorescence variables measured. The main effect of cultivar was significant, however. Green switchgrass leaves had a higher P_n, F_v/F_m, Φ_{PSII}, qP, and ETR and a lower qN than red switchgrass leaves (Table 4.4).

P_n and qN in red and green coleus leaves were differentially affected by duration length. P_n in red coleus leaves decreased as exposure duration increased, and exposure durations of 2 h or more resulted in lower P_n than in untreated plants (Fig. 4.10). In green coleus leaves, none of the exposure durations were significantly different from untreated plants. Likewise, qN increased in red coleus leaves as exposure duration increased and durations of 2 h or greater led to significantly higher rates of qN than in untreated plants, whereas, qP in green coleus leaves was unaffected by exposure duration (data not shown).

ETR, qP, and Φ_{PSII} in coleus decreased as exposure duration increased, and an exposure duration of 4 h was significantly lower than a 0 h exposure duration (control)

(Table 4.5). Photochemical quenching (qP), across all durations, was greater in 'LifeLime' than in 'Big Red Judy' (0.91 and 0.89, respectively).

Discussion

Anthocyanins in coleus are localized in the upper and lower epidermis (and trichomes) of leaves and in the epidermal layer of petioles and stems. In switchgrass, anthocyanins are localized primarily in the upper epidermis, although they did appear in the lower epidermis under high irradiance (unpublished data). The location of anthocyanins suggests that their physiological function may be to provide photoprotection and help minimize the severity of damage caused by environmental stresses, particularly those that result in photoinhibition and have the potential to damage photosystems.

Of the four temperature/irradiance combinations examined, HT/LL was the least stressful to photosystems. As a result, higher rates of Φ_{PSII}, qP, and ETR were observed in this treatment relative to the other three treatments during the 4 d exposure duration. Net photosynthesis was lower, but this was because plants were exposed to a non-saturating irradiance. In this treatment, irradiance (150 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was not excessive and temperature (30 °C) did not limit enzyme functionality.

The HT/HL and LT/LL treatments resulted in stress responses in red and green leaves of both species, which can be attributed to the effect of high irradiance (600 $\mu mol \cdot m^{-2} \cdot s^{-1}$) in the HT/HL treatment and low temperature (10 °C) in the LT/LL

treatment. Values of Φ_{PSII} and qP were similar within each leaf color in coleus and switchgrass (Fig. 4.3 and 4.4), although the LT/LL treatment resulted in lower F_v/F_m and ETR than the HT/HL treatment during the 4 d exposure (Fig. 4.1 and 4.6).

The LT/HL treatment was the most stressful environment, and resulted in the lowest F_v/F_m, P_n, Φ_{PSII}, qP, and ETR throughout the duration exposure, regardless of species or leaf color, even after just 1 d exposure. F_v/F_m, P_n, and ETR continued to decrease as the exposure duration increased, while Φ_{PSII} and qP maintained constant, albeit very low, values as the exposure duration increased from 1 to 4 d. In this combination of low temperature and high irradiance, the two parameters appeared to enhance the deleterious effects of the other on photosystem functionality and carbon fixation. Low temperatures can decrease enzyme activity in the Calvin Cycle, resulting in an excess pool of reduced cofactors, and decreased membrane fluidity, which impairs electron transport. High irradiance results in the absorption of more photons than can be effectively utilized in photochemistry (Demmig-Adams and Adams, 1992; Krause, 1988; Manetas, 2006).

Studies have shown that chilling, in combination with high irradiance, is more deleterious than chilling at low irradiance or in the dark. In the dark, a decrease from 22 °C to 7 °C did not affect F_v/F_m of rye, pea, maize, or cucumber, but at 520 $\mu mol \cdot m^{-2} \cdot s^{-1}$, F_v/F_m declined to less than 20% of the initial value in all four species (Feierabend et al., 1992). Janda et al. (1996) observed that *Zea mays* plants exposed to chilling temperatures had a minimal depression in F_v/F_m when returned to ambient temperatures in the dark but a greater depression when returned to ambient temperatures in the light.

In *Sorghum* NK145, the depression in P_n due to chilling injury increased as irradiance increased and the percent recovery of initial P_n was lower (Taylor and Rowley, 1971). In our study, high irradiance also enhanced the effects of low temperature, but the presence of anthocyanins helped mitigate some of the effects of the LT/HL exposure, more so coleus than in switchgrass.

To provide a photoprotective role in leaves, anthocyanins should help reduce the severity of damage from temperature and light stress and help leaves recover more quickly following cessation of the stress event, as we saw in coleus here (Fig. 4.1A and B). The ability of anthocyanins to help minimize photoinhibition has been noted for other species as well. Anthocyanic and anthocyan-deficient *V. elliottii* had similar F_v/F_m ratios in a low stress environment, but anthocyanic leaves maintained a higher F_v/F_m during a 5 d stress and subsequent recovery in a high stress environment (Hoch et al., 2003). Gould et al. (2010) observed that red-stemmed selections of five species exhibited less of a decrease in F_v/F_m than green stems following low temperature, high light stress, and Smillie and Hetherington (1999) observed higher F_v/F_m ratios in purple *Bauhinia variegata* pods relative to green pods in response to a short (75 min) low temperature, high irradiance stress. In high light stress, Hughes et al. (2010) noted less of a decline in F_v/F_m in red *Galax urceolata* leaves (55% decrease) than in green leaves (86% decrease), and red leaves recovered more quickly (1 d for red, 5 d for green). Manetas et al. (2002) also noted a photoprotective role for anthocyanins, as young, red *Rosa* and *Ricinus communis* L. leaves experienced less of a decline in F_v/F_m following a high light exposure than mature, green leaves.

While anthocyanins helped minimize a decline in F_v/F_m in coleus and reduced the recovery interval, red and green switchgrass did not exhibit differential reductions in F_v/F_m after a 4 d LT/HL exposure and had similar recovery intervals (Fig. 4.1C and D). This is similar to results reported by Zhang et al. (2011), in which only minimal support for a photoprotective role was found in red *Begonia semperflorens* leaves following a 1 d high light stress at ambient temperature. It is possible, however, that a greater difference between red and green *B. semperflorens* leaves may have occurred under a high light, low temperature exposure.

Photoinhibition of P_n was reduced in red coleus and switchgrass leaves, relative to green leaves. Net photosynthesis decreased only 58% in red coleus but 100% in green coleus after a 4 d exposure, and P_n decreased 32% in red switchgrass and 78% in green switchgrass. Recovery time was similar for red and green leaves, however (within 2 d of return to ambient conditions). Although red switchgrass leaves had a lower P_n than green leaves under pre-stress, stress, and recovery conditions, the percent reduction in P_n in red leaves was lower in magnitude. The presence of anthocyanins likely resulted in lower light capture by underlying chloroplasts (Pietrini et al., 2002), so while they provided a photoprotective function (a lower reduction in P_n) during LT/HL, it still was not great enough to have similar or higher P_n than green leaves at the LT/HL exposure.

In coleus and switchgrass, red leaves had a lower P_n under ambient conditions than green leaves, similar to results reported for a number of species (Barker et al., 1997; Gould et al., 2002; Tuohy and Choinski, 1990; Woodall et al., 1998; Zhang et al., 2011). However, red coleus leaves did have a higher P_n during the LT/HL stress and a lower

percent reduction in P_n than green leaves, two pieces of evidence in favor of a photoprotective role for anthocyanins in coleus.

Decreases in Φ_{PSII} were relatively consistent across both species and leaf colors after a 4 d stress (LT/HL). Values were 0.06 in red and green switchgrass and green coleus and 0.14 in red coleus. Effective quantum yield decreased dramatically within the first day of LT/HL exposure, and recovered within 2 d of return to ambient. A lack of differences between red and green leaves in either species is similar to results obtained for red and green zonal geranium (*Pelargonium* x *hortorum*) following low temperature exposure (4 °C for 30 min) (Liakopoulos and Spanorigas, 2012), but stands in contrast to differences observed in other species. In *Cornus stolonifera*, red leaves recovered from a 30 min exposure to high-intensity (1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$) white or blue light, but yellow-senescing leaves did not fully recover, even after a 6 h in darkness (Feild et al., 2001). Red *Zea mays* leaves had a higher Φ_{PSII} than green leaves following cold temperature, high light stress, even after a 2 h recovery (Pietrini et al., 2002).

Anthocyanins in coleus or switchgrass leaves did not provide additional protection against UV-B exposure. ETR, qP, and Φ_{PSII} decreased as exposure duration increased in coleus, with red and green leaves responding similarly. This is in contrast to the results from Burger and Edwards (1996), who suggested that anthocyanins may be more beneficial in providing protection against UV-B radiation rather than excess visible light. Their work however, involved different coleus cultivars.

Switchgrass leaves were unaffected by supplemental UV-B exposure at the fluence rate (16 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and exposure durations (< 24 h) used in our study. This

grass does have a waxy cuticle, which may explain their lack of response to UV-B radiation. UV-B penetration can range from 0% to 40% (Teramura and Sullivan, 1994), and greater penetration to the epidermis may result in increased leaf damage. The presence of a thick, waxy cuticle is just one mechanism for increased UV-B protection, and it appears to be beneficial in switchgrass. In a follow-up experiment, continual UV-B exposure of switchgrass plants for up to 2 weeks failed to elicit any change in anthocyanin content, leaf P_n or chlorophyll fluorescence (data not shown). Red and green coleus leaves, however, became visibly paler in color within 48 h of continued UV-B exposure.

Photoprotection against low temperature and high irradiance appeared to be a major function of anthocyanins in red coleus leaves. Distinctive advantages were observed in red leaves for F_v/F_m and P_n but not for Φ_{PSII}, qP, or ETR, indicating that anthocyanins may serve a greater photoprotective role in dark-adapted, but not in light-adapted leaves. Light-adapted green coleus leaves were likely able to upregulate other protective mechanisms in response to the LT/HL exposure and compensate for the lack of anthocyanins, resulting in similar responses to a short-term (1 to 4 d) light and temperature stress. In red leaves, due to the localization of anthocyanins in the epidermis, it is likely that they provide a light screen to limit the capture of excess photons by chloroplasts. However, it is also possible that anthocyanins may simply be correlated with other factors conferring increased stress tolerance.

Anthocyanins provided a benefit to coleus but not switchgrass, which may be due to several reasons. Switchgrass may have other mechanisms to help mitigate excess

irradiation (ultraviolet and visible light) and anthocyanins may simply serve as a "back-up" to these other mechanisms, or plants may not have been exposed to a severe enough stress to generate differences between red and green leaves. Switchgrass leaves have a long, narrow leaf morphology, an adaptation to a high-light environment, and coleus have a large, flat, horizontal leaf surface characteristic of understory species. Shade plants, in general, have a greater risk of photoinhibition than sun plants (Demmig-Adams, 1992; Krause, 1988). Switchgrass is also adapted to a temperate climate (whereas coleus originates from a tropical climate) and can withstand lower temperatures than coleus before inducement of chilling injury. The combination of these attributes may have allowed red and green switchgrass leaves to have similar responses at the selected temperatures and irradiances than coleus leaves.

In addition, coleus leaves are constitutively red or green and anthocyanic leaves are red during leaf expansion, maturity, and senescence. They have a baseline anthocyanin content at all times and the intensity will increase or decrease based on changes in environmental conditions and internal signals. Mature switchgrass leaves may be completely green, partially red, or completely red (cultivar dependent). Ruby Ribbons^{TM} plants have both red and green leaves and more leaves will develop pigmentation in response to high light intensities (Boldt et al., 2011a). The development of anthocyanins in switchgrass leaves may be a mechanism to protect against further stress, but may not give them an advantage relative to green leaves. If so, this would be similar to the response observed in *Cistus creticus*, in which anthocyanins provide some

protection to leaves that are already predisposed to photodamage, but do not provide an advantage relative to green leaves (Zeliou et al., 2009).

It should not be overlooked that human selection of red and purple ornamental plants for commercial sale has been taking place for many years. The anthocyanic coleus cultivar in this study ('Big Red Judy') was selected under full sun conditions (D.G. Clark, personal communication), so it may have other built in advantages for high light in addition to the presence of anthocyanins. Switchgrass cultivars selected to have increased red coloration in landscapes often have lower growth rates than green-leaved selections. 'Heavy Metal' is the maternal parent of Ruby Ribbons^{TM}, a cultivar released by Mark Brand (US plant patent US17,944 P3, granted 2007). 'Heavy Metal' has a more vigorous growth habit than Ruby Ribbons^{TM} in field and greenhouse experiments (personal observation). It is highly possible that selecting red foliage is beneficial aesthetically but not physiologically.

In summary, we found that anthocyanins provided a photoprotection role against excess irradiance at low, but not high, temperature in coleus but not switchgrass. No protection against supplemental UV-B at low irradiance was found in either species. Anthocyanin presence resulted in a decrease in the severity of low temperature, high-light stress, as quantified by F_v/F_m and P_n. Red coleus leaves recovered faster after cessation of the LT/HL stress. Anthocyanin presence may be beneficial in selection of cold-tolerant cultivars of tropical species that can survive occasional exposure to cool temperatures and high light.

Table 4.1. Mean greenhouse air temperatures (°C) and daily light integral (DLI, mol·m^{-2}·d^{-1}) for coleus (cutting production, propagation, and finished production) and switchgrass (finished production) in Expt. 1 and 2. Expt. 1 was repeated twice (Spring and Fall 2011). Values are mean ± SD.

Expt.	Season	Growth stage	Air Temperature (°C)		DLI
			Day	Night	(mol·m^{-2}·d^{-1})
Expt. 1	Spring 2011	Cutting formation	23.2 ± 0.9	16.7 ± 0.2	22.3 ± 5.9
		Propagation	20.9 ± 2.4	17.5 ± 0.3	19.1 ± 5.8
		Finished production	24.4 ± 3.7	20.1 ± 2.7	17.9 ± 6.2
	Fall 2011	Cutting formation	29.0 ± 2.7	25.3 ± 2.5	14.3 ± 2.5
		Propagation	27.7 ± 1.4	24.2 ± 1.6	14.5 ± 2.0
		Finished production	24.8 ± 2.8	21.4 ± 2.7	13.0 ± 2.2
Expt. 2	Spring 2012	Cutting formation	14.3 ± 2.1	22.3 ± 0.8	17.1 ± 0.3
		Propagation	18.4 ± 2.9	22.9 ± 0.6	16.8 ± 0.3
		Finished production	21.3 ± 6.1	23.1 ± 0.8	16.7 ± 0.2

Table 4.2. Analysis of variance for red and green *Solenostemon scutellarioides* (coleus) and *Panicum virgatum* (switchgrass) cultivars (Cv) transferred from ambient conditions (20 °C, 300 µmol·m^{-2}·s^{-1}) to four temperature and irradiance treatments (Trt) for 4 d, followed by a return to ambient conditions for a 4 d recovery (Day). The experimental layout was a split plot, with treatment (Trt) as the main plot, cultivar (Cv) as the sub-plot, and repeated measures (Day) on the sub-plot.

		Variable						
Species	Source	F_v/F_m[z]	P_n	F_v'/F_m'	Φ_{PSII}	qP	qN	ETR
Coleus	Rep	0.28[y]	0.66	0.30	0.47	0.56	0.99	0.33
	Trt	<0.0001	0.008	<0.0001	0.0001	0.0001	0.01	0.0001
	Cv	<0.0001	0.44	0.005	0.70	0.46	0.005	0.55
	Trt x Cv	<0.0001	0.20	0.002	0.07	0.24	0.66	0.10
	Day	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Day x Trt	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Day x Cv	<0.0001	0.002	<0.0001	0.23	0.14	0.005	0.25
	Day x Trt x Cv	<0.0001	0.14	0.0007	0.42	0.23	0.20	0.56
Switchgrass	Rep	0.61	0.14	0.67	0.25	0.10	0.62	0.26
	Trt	0.04	0.04	0.004	0.0002	0.004	0.004	0.002
	Cv	0.004	<0.0001	0.0008	0.0003	0.003	0.002	<0.0001
	Trt x Cv	0.12	0.11	0.13	0.04	0.02	0.08	0.006
	Day	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.01	<0.0001
	Day x Trt	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Day x Cv	0.02	<0.0001	0.03	0.15	0.47	0.17	0.16
	Day x Trt x Cv	0.72	<0.0001	0.21	0.11	0.03	0.32	0.003

[z] Abbreviations: maximum quantum yield (dark-adapted leaves), F_v/F_m; net photosynthetic rate (µmol CO_2·m^{-2}·s^{-1}), Pn; maximum quantum yield (light-adapted leaves), F_v'/F_m'; effective quantum yield, Φ_{PSII}; photochemical quenching, qP; non-photochemical quenching, qN; electron transport rate (µmol·m^{-2}·s^{-1}), ETR

[y] Significant if $P < 0.05$.

Table 4.3. Analysis of variance for *Solenostemon scutellarioides* (coleus) and *Panicum virgatum* (switchgrass) exposed to short-term supplemental UV-B radiation (16 µmol·m^{-2}·s^{-1}).

		Variable					
Species	Source	P_n[z]	F_v/F_m	Φ_{PSII}	qP	qN	ETR
Coleus	Cultivar	0.007[y]	0.15	0.40	0.02	0.92	0.31
	Duration	0.02	0.33	0.01	0.03	0.002	0.008
	Cv x Dur	0.0009	0.59	0.07	0.18	0.005	0.07
Switchgrass	Cultivar	<0.0001	0.03	<0.0001	<0.0001	<0.0001	<0.0001
	Duration	0.79	0.61	0.95	0.94	0.33	0.95
	Cv x Dur	0.12	0.96	0.20	0.29	0.42	0.20

[z] Abbreviations: net photosynthetic rate (µmol CO_2·m^{-2}·s^{-1}), Pn; maximum quantum yield (dark-adapted leaves), F_v/F_m; maximum quantum yield (light-adapted leaves), F_v'/F_m'; effective quantum yield, Φ_{PSII}; photochemical quenching, qP; non-photochemical quenching, qN; electron transport rate (µmol·m^{-2}·s^{-1}), ETR

[y] Significant at $P < 0.05$.

Table 4.4. Photosynthesis and chlorophyll fluorescence parameters in *Panicum virgatum* (switchgrass) following exposure to supplemental UV-B radiation (0 to 8 h) at 150 µmol·m^{-2}·s^{-1} irradiance. Values (mean ± $_{SE}$) are pooled across UV-B duration.

	Variable					
Cultivar	P_n	F_v/F_m	Φ_{PSII}	qP	qN	ETR
'Heavy Metal'	7.90 ± 0.36 a[y]	0.70 ± 0.00 a	0.54 ± 0.01 a	0.94 ± 0.01 a	0.35 ± 0.02 b	44.1 ± 0.8 a
Ruby Ribbons™	2.15 ± 0.34 b	0.68 ± 0.01 b	0.33 ± 0.02 b	0.72 ± 0.03 b	0.62 ± 0.04 a	26.9 ± 1.8 b

[z] Abbreviations: net photosynthetic rate (µmol CO_2·m^{-2}·s^{-1}), Pn; maximum quantum yield (dark-adapted leaves), F_v/F_m; effective quantum yield, Φ_{PSII}; photochemical quenching, qP; non-photochemical quenching, qN; electron transport rate (µmol·m^{-2}·s^{-1}), ETR

[y] Values in each column followed by different letters are significant at $P<0.05$ (Tukey's HSD).

Table 4.5. Influence of supplemental UV-B exposure on effective quantum yield (Φ_{PSII}), photochemical quenching (qP), and nonphotochemical quenching (qN) in *Solenostemon scutellarioides* (coleus). Values (mean ± $_{SE}$) at each duration are pooled across cultivar.

	Variable		
Duration	Φ_{PSII}	qP	ETR
0	0.71 a[z]	0.92 a	58 a
1	0.67 ab	0.90 ab	55 ab
2	0.66 ab	0.90 ab	54 ab
4	0.62 b	0.87 b	51 b
$HSD_{\alpha=0.05}$	0.06	0.05	5

[z] Values in each column followed by different letters are significant at $P<0.05$ (Tukey's HSD).

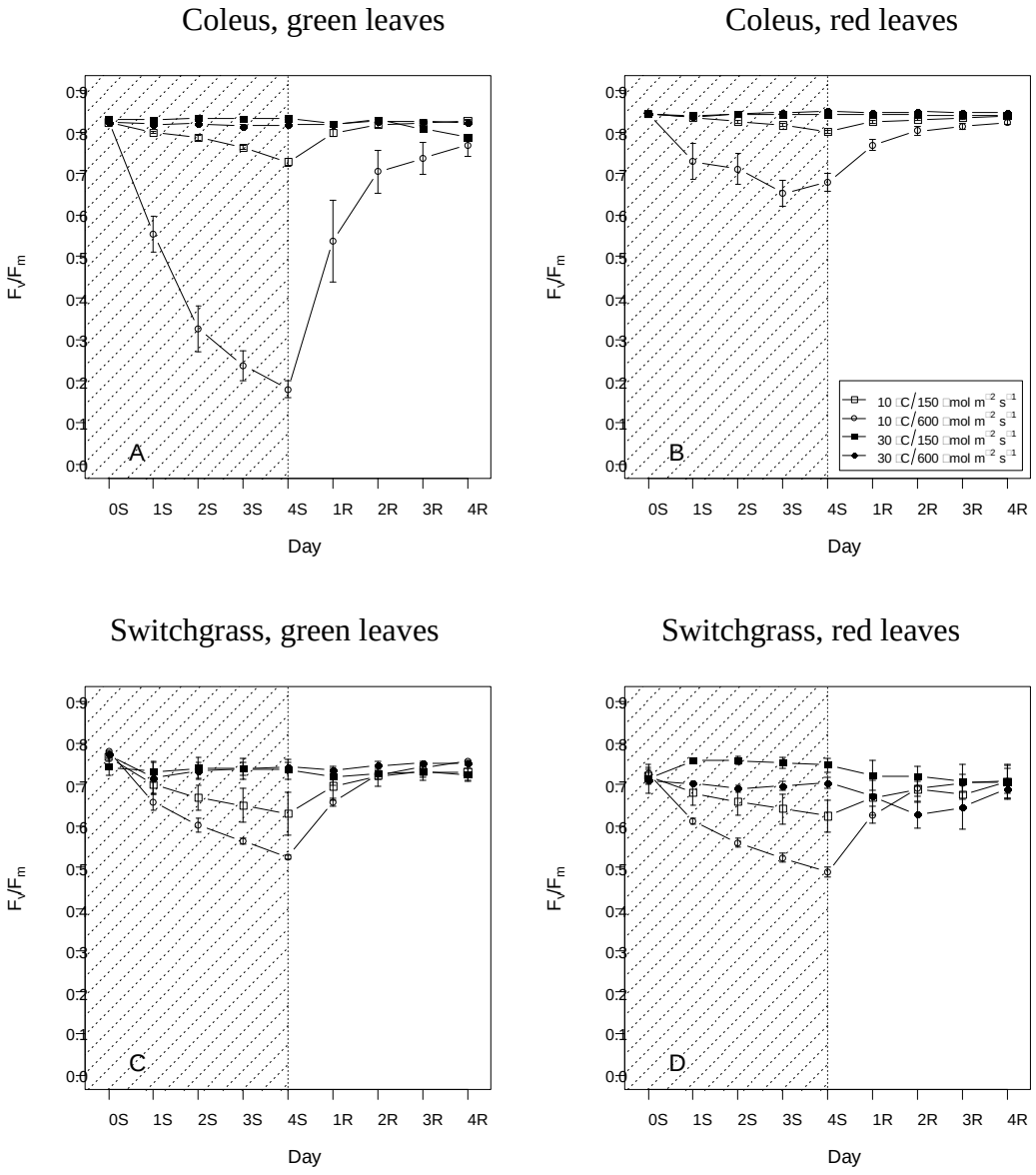

Fig 4.1. Maximum quantum yield (F_v/F_m) of dark-adapted green (A and C) and red (B and D) leaves of coleus (A and B) and switchgrass (C and D). Plants were transferred from ambient conditions (0S; 20 °C, 300 µmol·m^{-2}·s^{-1} *PAR*) to various temperature and irradiance combinations for a 4 d 'stress' (1S-4S), then returned to ambient conditions for a 4 d 'recovery' (1R-4R): A) coleus 'LifeLime' (green leaves), B) coleus 'Big Red Judy' (red), C) switchgrass 'Heavy Metal' (green), and D) switchgrass Ruby Ribbons^{TM} (red). In each graph, treatments are denoted with the following symbols: low temperature/low light (□; 10 °C, 150 µmol·m^{-2}·s^{-1}), low temperature/high light (○; 10 °C, 600 µmol·m^{-2}·s^{-1}), high temperature/low light (■; 30 °C, 150 µmol·m^{-2}·s^{-1}), and high temperature/high light (●; 30 °C, 600 µmol·m^{-2}·s^{-1}).

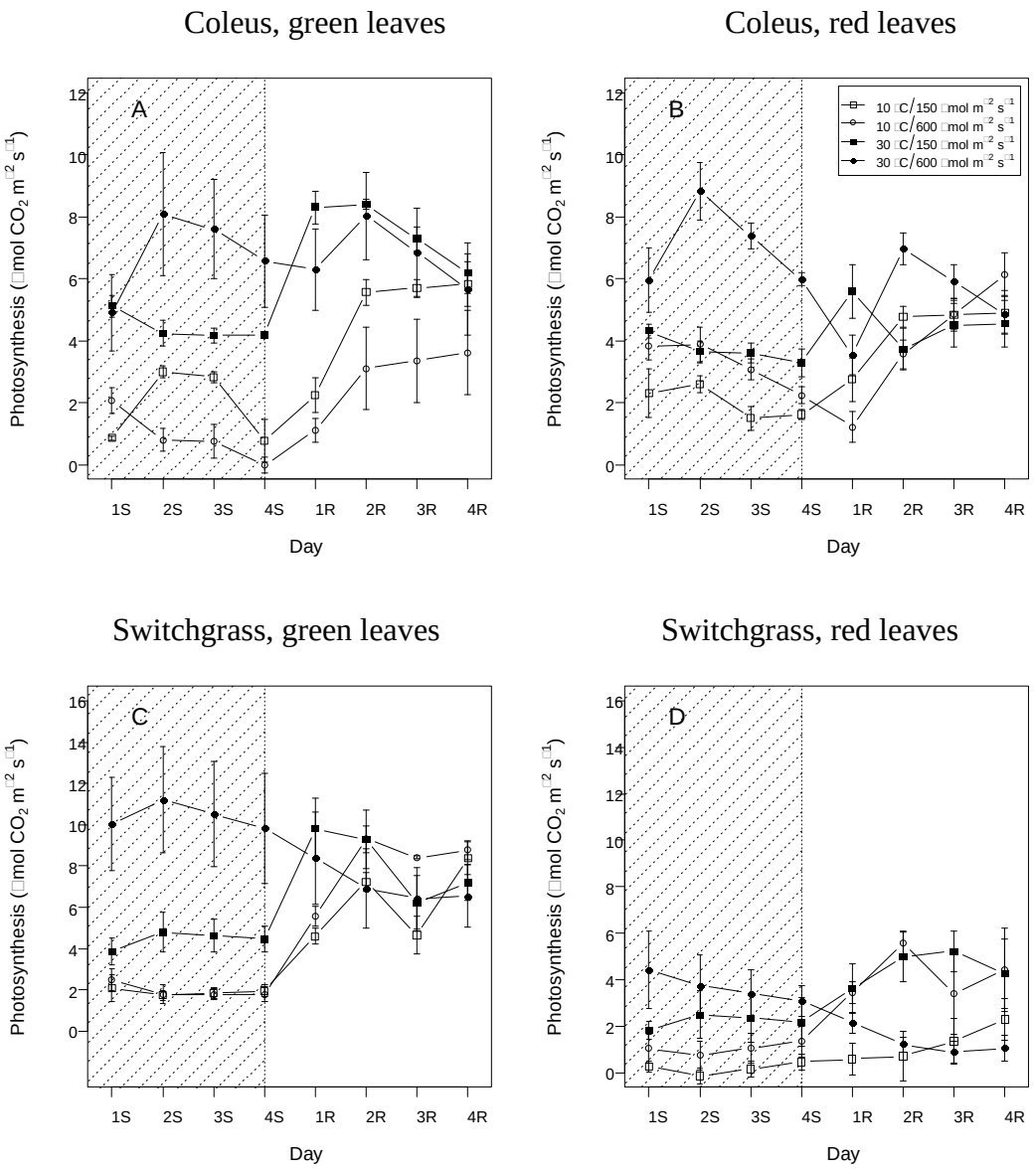

Fig. 4.2. Net photosynthetic rate (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) for green (A and C) and red (B and D) leaves of coleus (A and B) and switchgrass (C and D). Plants were transferred from ambient conditions (0S; 20 °C, 300 μmol·m^{-2}·s^{-1} *PAR*) to various temperature and irradiance combinations for a 4 d 'stress' (1S-4S), then returned to ambient conditions for a 4 d 'recovery' (1R-4R): A) coleus 'LifeLime' (green leaves), B) coleus 'Big Red Judy' (red), C) switchgrass 'Heavy Metal' (green), and D) switchgrass Ruby Ribbons™ (red). In each graph, treatments are denoted with the following symbols: low temperature/low light (□; 10 °C, 150 μmol·m^{-2}·s^{-1}), low temperature/high light (○; 10 °C, 600 μmol·m^{-2}·s^{-1}), high temperature/low light (■; 30 °C, 150 μmol·m^{-2}·s^{-1}), and high temperature/high light (●; 30 °C, 600 μmol·m^{-2}·s^{-1}).

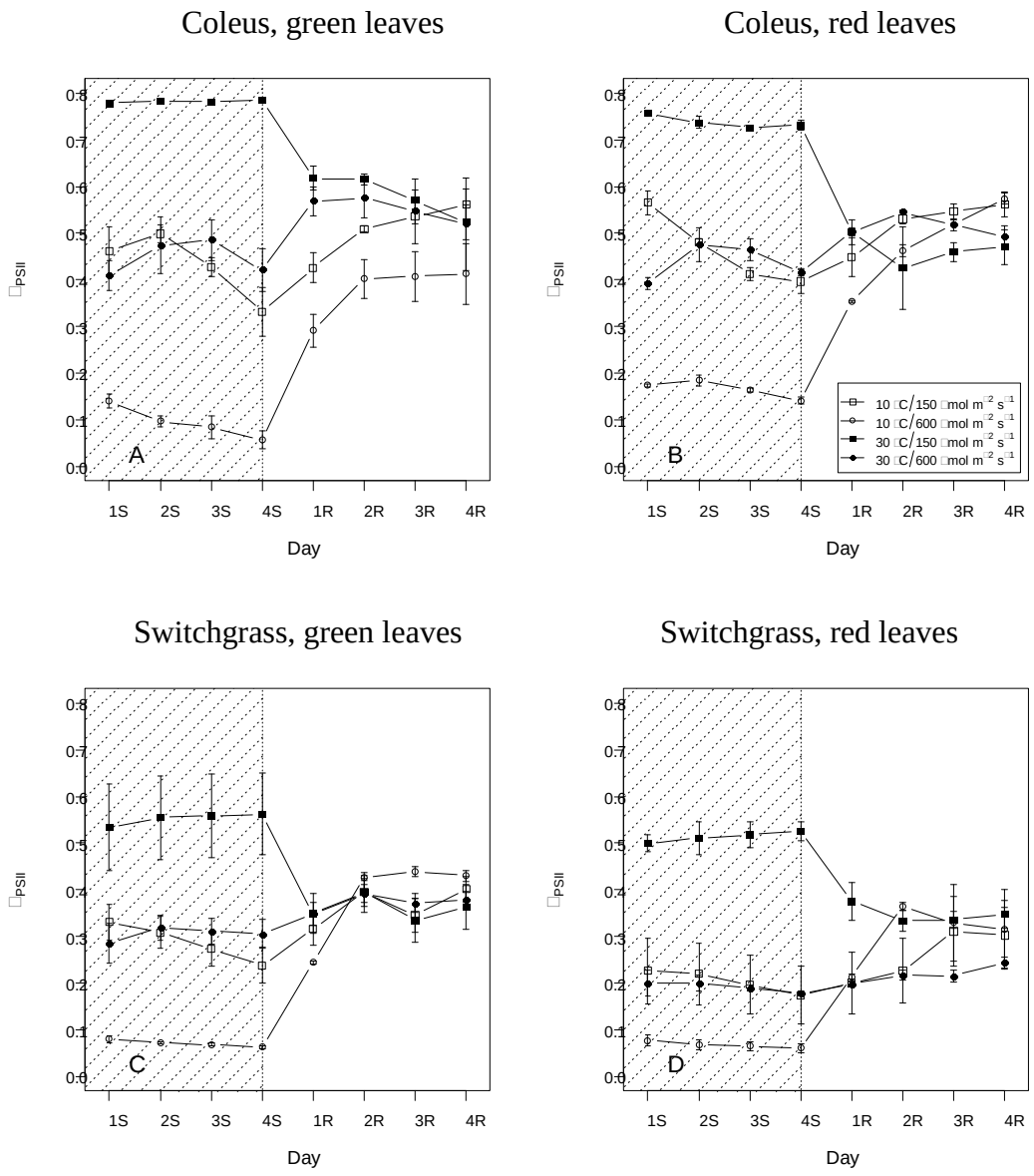

Fig. 4.3. Effective quantum yield (Φ_{PSII}) of green (A and C) and red (B and D) leaves of coleus (A and B) and switchgrass (C and D). Plants were transferred from ambient conditions (0S; 20 °C, 300 µmol·m^{-2}·s^{-1} *PAR*) to various temperature and irradiance combinations for a 4 d 'stress' (1S-4S), then returned to ambient conditions for a 4 d 'recovery' (1R-4R): A) coleus 'LifeLime' (green leaves), B) coleus 'Big Red Judy' (red), C) switchgrass 'Heavy Metal' (green), and D) switchgrass Ruby Ribbons^{TM} (red). In each graph, treatments are denoted with the following symbols: low temperature/low light (□; 10 °C, 150 µmol·m^{-2}·s^{-1}), low temperature/high light (○; 10 °C, 600 µmol·m^{-2}·s^{-1}), high temperature/low light (■; 30 °C, 150 µmol·m^{-2}·s^{-1}), and high temperature/high light (●; 30 °C, 600 µmol·m^{-2}·s^{-1}).

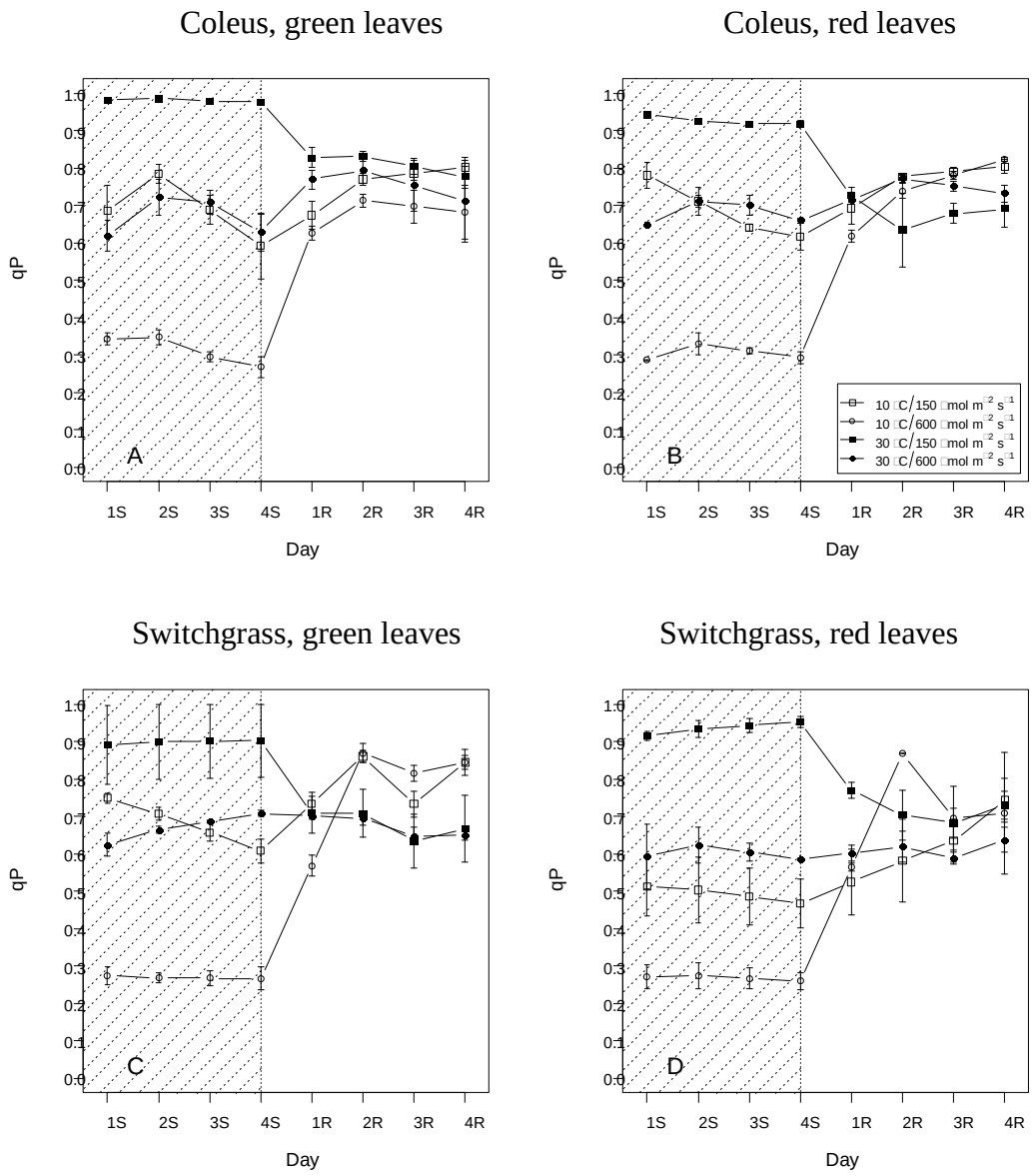

Fig. 4.4. Photochemical quenching (qP) for green (A and C) and red (B and D) leaves of coleus (A and B) and switchgrass (C and D). Plants were transferred from ambient conditions (0S; 20 °C, 300 µmol·m^{-2}·s^{-1} *PAR*) to various temperature and irradiance combinations for a 4 d 'stress' (1S-4S), then returned to ambient conditions for a 4 d 'recovery' (1R-4R): A) coleus 'LifeLime' (green leaves), B) coleus 'Big Red Judy' (red), C) switchgrass 'Heavy Metal' (green), and D) switchgrass Ruby Ribbons^{TM} (red). In each graph, treatments are denoted with the following symbols: low temperature/low light (□; 10 °C, 150 µmol·m^{-2}·s^{-1}), low temperature/high light (○; 10 °C, 600 µmol·m^{-2}·s^{-1}), high temperature/low light (■; 30 °C, 150 µmol·m^{-2}·s^{-1}), and high temperature/high light (●; 30 °C, 600 µmol·m^{-2}·s^{-1}).

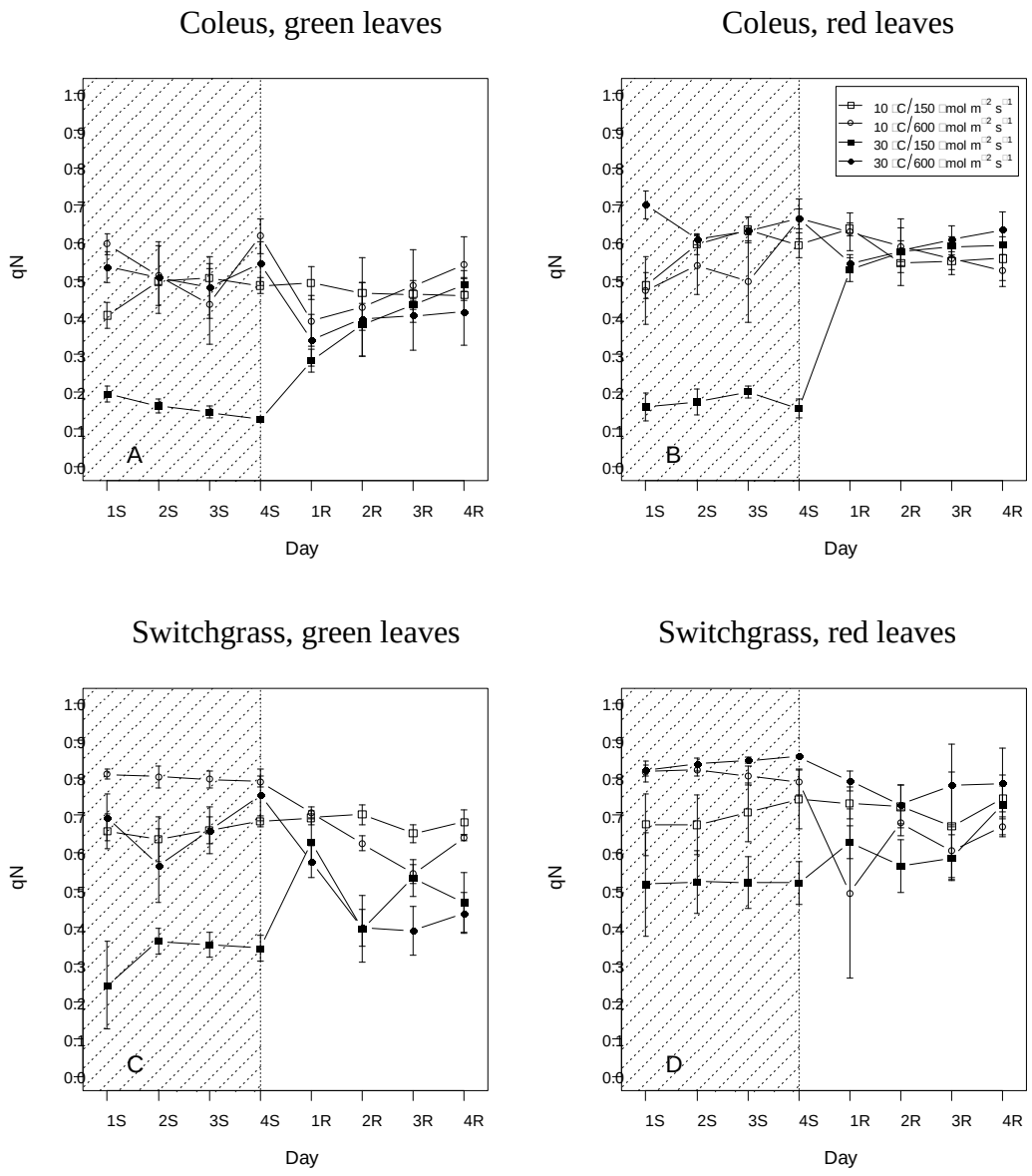

Fig. 4.5. Non-photochemical quenching (qN) for green (A and C) and red (B and D) leaves of coleus (A and B) and switchgrass (C and D). Plants were transferred from ambient conditions (0S; 20 °C, 300 µmol·m^{-2}·s^{-1} *PAR*) to various temperature and irradiance combinations for a 4 d 'stress' (1S-4S), then returned to ambient conditions for a 4 d 'recovery' (1R-4R): A) coleus 'LifeLime' (green leaves), B) coleus 'Big Red Judy' (red), C) switchgrass 'Heavy Metal' (green), and D) switchgrass Ruby Ribbons^{TM} (red). In each graph, treatments are denoted with the following symbols: low temperature/low light (□; 10 °C, 150 µmol·m^{-2}·s^{-1}), low temperature/high light (○; 10 °C, 600 µmol·m^{-2}·s^{-1}), high temperature/low light (■; 30 °C, 150 µmol·m^{-2}·s^{-1}), and high temperature/high light (●; 30 °C, 600 µmol·m^{-2}·s^{-1}).

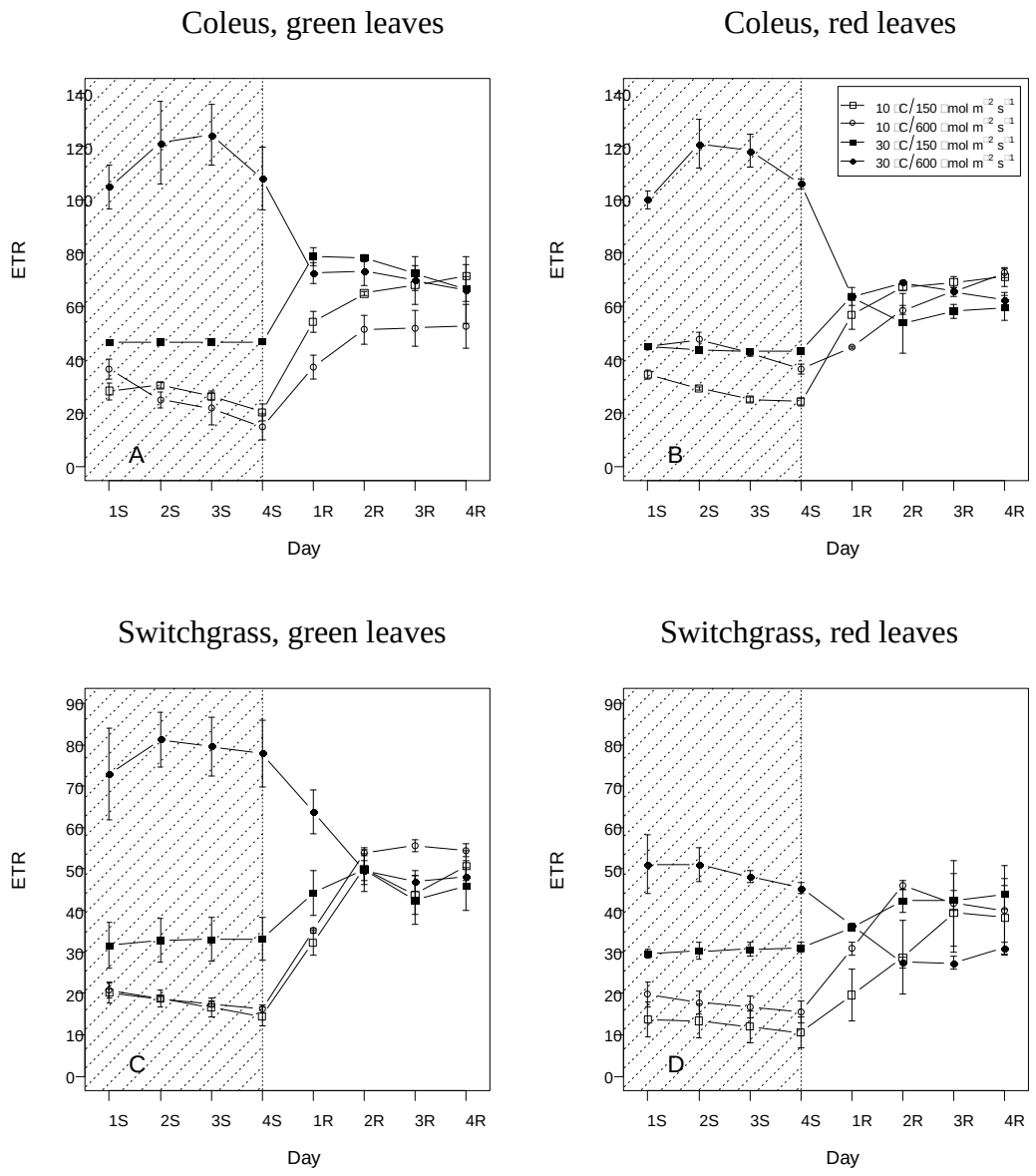

Fig. 4.6. Electron transport rate (ETR, $\mu mol \cdot m^{-2} \cdot s^{-1}$) for green (A and C) and red (B and D) leaves of coleus (A and B) and switchgrass (C and D). Plants were transferred from ambient conditions (0S; 20 °C, 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PAR*) to various temperature and irradiance combinations for a 4 d 'stress' (1S-4S), then returned to ambient conditions for a 4 d 'recovery' (1R-4R): A) coleus 'LifeLime' (green leaves), B) coleus 'Big Red Judy' (red), C) switchgrass 'Heavy Metal' (green), and D) switchgrass Ruby Ribbons™ (red). In each graph, treatments are denoted with the following symbols: low temperature/low light (□; 10 °C, 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$), low temperature/high light (○; 10 °C, 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$), high temperature/low light (■; 30 °C, 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$), and high temperature/high light (●; 30 °C, 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$).

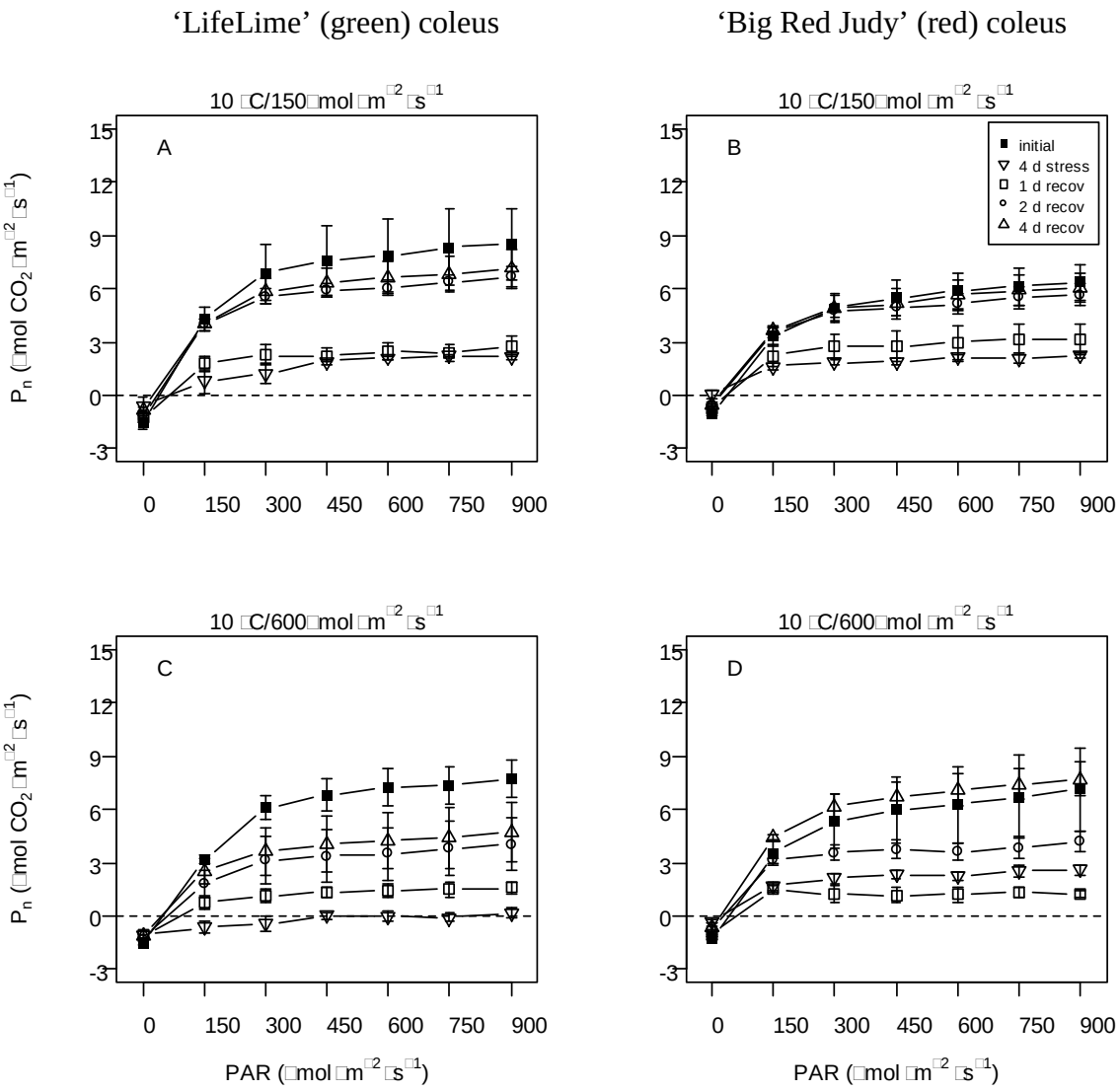

Fig. 4.7. Photosynthetic irradiance response curves for 'LifeLime' (green; A and C) and 'Big Red Judy' (red; B and D) coleus leaves following exposure to 10 °C for 4 d at low (150 µmol·m^{-2}·s^{-1}; A and B) or high (600 µmol·m^{-2}·s^{-1}; C and D) irradiance, followed by recovery at ambient temperature (20 °C) and irradiance (300 µmol·m^{-2}·s^{-1}) for 4 d. Net photosynthesis (µmol CO_2·m^{-2}·s^{-1}) was determined before the start of low temperature exposure, after 4 d low temperature exposure, and after 1, 2, and 4 d recovery at ambient conditions.

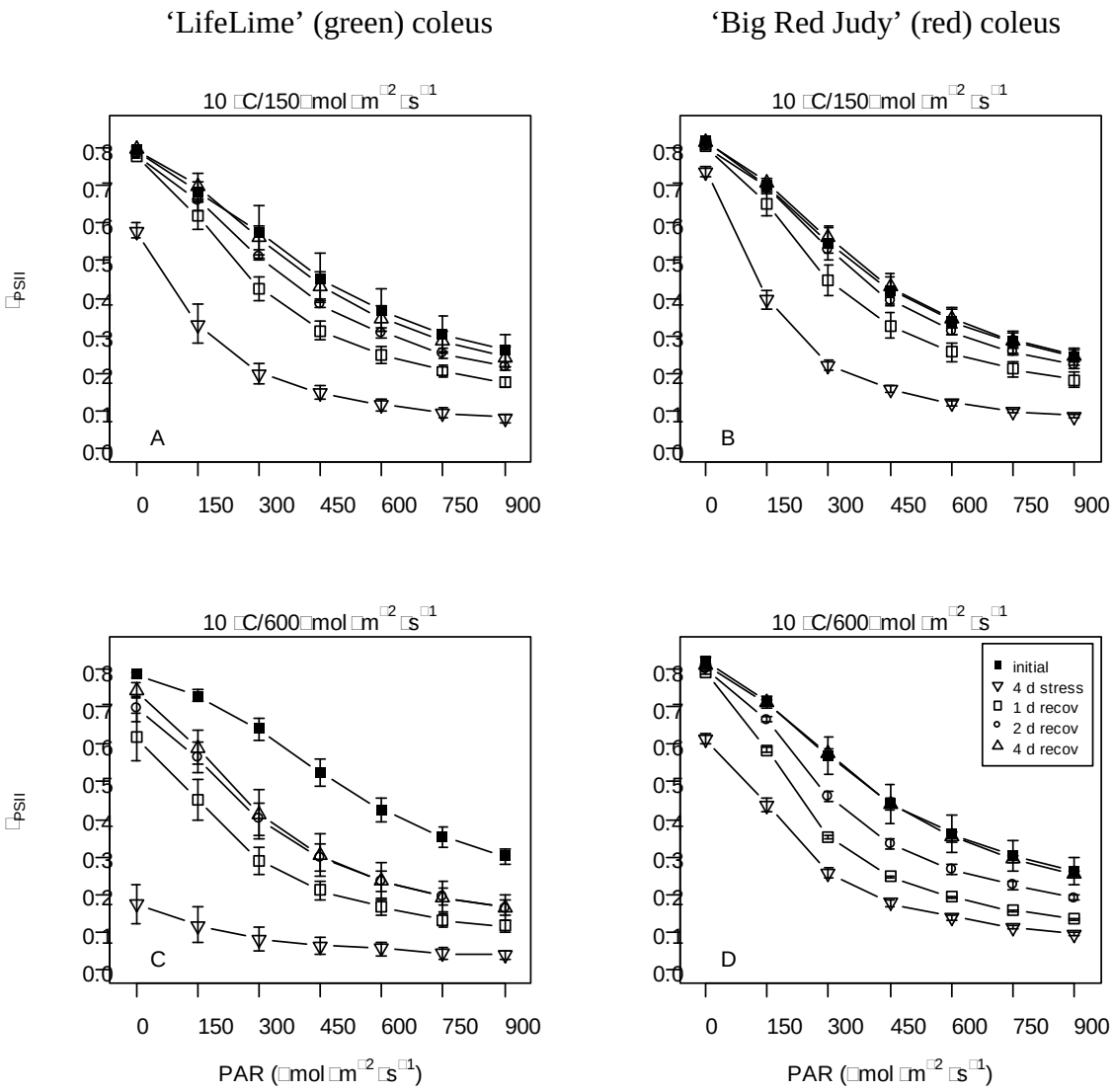

Fig. 4.8. Effective quantum yield (Φ_{PSII}) in response to irradiance of 'LifeLime' (green; A and C) and 'Big Red Judy' (red; B and D) coleus leaves following exposure to 10 °C for 4 d at low (150 µmol·m^{-2}·s^{-1}; A and B) or high (600 µmol·m^{-2}·s^{-1}; C and D) irradiance, followed by recovery at ambient temperature (20 °C) and irradiance (300 µmol·m^{-2}·s^{-1}) for 4 d. Net photosynthesis (µmol CO_2·m^{-2}·s^{-1}) was determined before the start of low temperature exposure, after 4 d low temperature exposure, and after 1, 2, and 4 d recovery at ambient conditions.

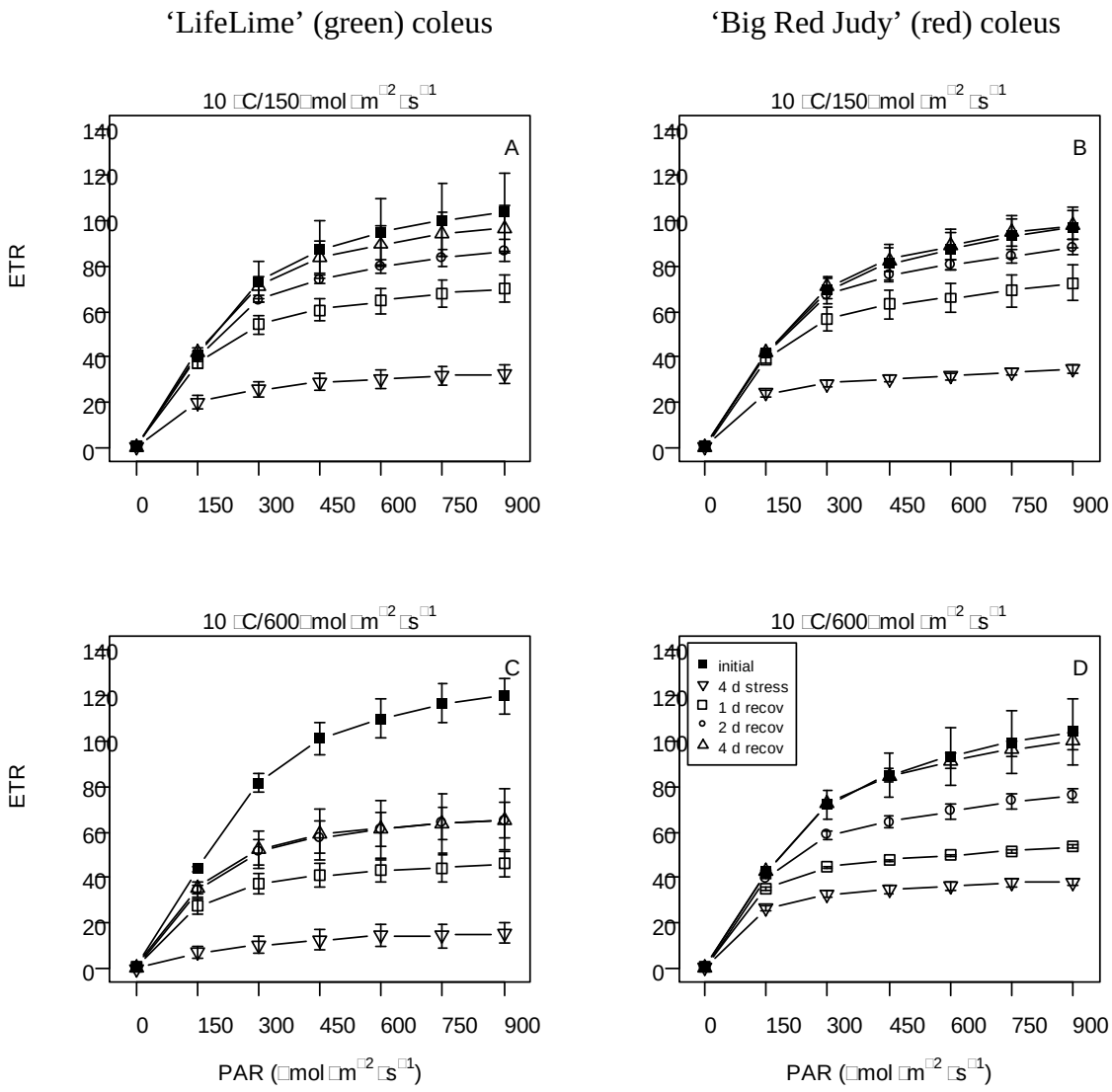

Fig. 4.9. Electron transport rate (ETR, $\mu mol \cdot m^{-2} \cdot s^{-1}$) in response to irradiance of 'LifeLime' (green; A and C) and 'Big Red Judy' (red; B and D) coleus leaves following exposure to 10 °C for 4 d at low (150 $\mu mol \cdot m^{-2} \cdot s^{-1}$; A and B) or high (600 $\mu mol \cdot m^{-2} \cdot s^{-1}$; C and D) irradiance, followed by recovery at ambient temperature (20 °C) and irradiance (300 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for 4 d. Net photosynthesis ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$) was determined before the start of low temperature exposure, after 4 d low temperature exposure, and after 1, 2, and 4 d recovery at ambient conditions.

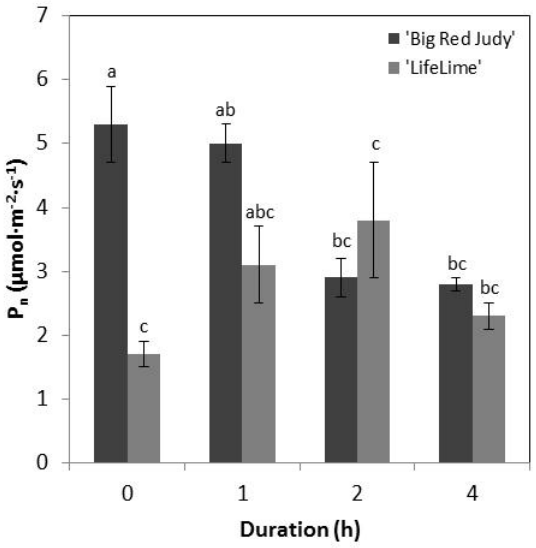

Fig. 4.10. Net photosynthetic rate (P_n; µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) of red ('Big Red Judy') and green ('LifeLime') coleus leaves in response to supplemental UV-B exposure for varying durations (0 to 4 h). Values are mean ± $_{SE}$ (n=3) and bars with different letters are significantly different at $P<0.05$ (Tukey's HSD).

Chapter 5

Temperature and Irradiance Affect Foliar Anthocyanin Content of *Panicum virgatum*, *Pennisetum advena*, and *Solenostemon scutellarioides*

Solenostemon scutellarioides L. (Codd) (coleus), *Panicum virgatum* L. (switchgrass), and *Pennisetum advena* Wipff & Veldkamp (purple fountaingrass) are cultivated for their ornamental color. Irradiance and temperature, but not UV-B or low P, primarily increased anthocyanin content in red coleus. Only irradiance influenced anthocyanin content in switchgrass and purple fountaingrass. Anthocyanins in coleus were present in the upper and lower epidermis and trichomes. In purple fountaingrass and red switchgrass, anthocyanins localized in the upper epidermis and sometimes also accumulated in the lower epidermis (following exposure to high irradiance). Foliar anthocyanin content was greatest in purple fountaingrass leaves exposed to 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and in Ruby Ribbons^{TM} switchgrass leaves exposed to 300 and 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Percent anthocyanic leaves increased, from 0%, 10%, and 18% ('Heavy Metal' and Ruby Ribbons^{TM} switchgrass and purple fountaingrass, respectively) at 75 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 17%, 83%, and 100% at 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. In all red-leaved coleus ('Big Red Judy', 'Dark Star', 'Dipt in Wine', 'Royal Glissade', 'Sedona', 'Solar Red', 'Twist and Twirl' red, 'Versa Burgundy to Green', and 'Versa Crimson Gold'), anthocyanin content increased in response to increased irradiance. The magnitude of the response was cultivar specific, and 'Big Red Judy', 'Royal Glissade', and 'Twist and Twirl' red had the

greatest increases in anthocyanin content (>700% after 20 d at 600 µmol·m^{-2}·s^{-1}). Chlorophyll content decreased in eight of 12 coleus cultivars studied as irradiance increased. In four coleus cultivars, photobleaching (loss of pigmentation) occurred at 600 µmol·m^{-2}·s^{-1} after 16 d. Changes in anthocyanin content as a result of increased irradiance could be observed after approximately 1 week in both coleus and grasses. Temperature (12 °C to 30 °C) influenced foliar anthocyanin content in coleus but not switchgrass, and the response was cultivar specific. Coleus 'Big Red Judy' and 'Twist and Twirl' red had maximum anthocyanin content at 12 °C, whereas anthocyanin content was lowest in 'Royal Glissade' at 12 °C. Differences in anthocyanin content resulting from temperature occurred after 15 d. Chlorophyll content in all coleus was lower at lower temperatures. To help improve red foliar pigmentation for improved aesthetic appeal at the retail level, growers can adjust the greenhouse environment of these species during the last 1 to 2 weeks of production to increase anthocyanin content.

Introduction

Leaf pigmentation often appears transiently in juvenile, mature, and senescing leaves (Close and Beadle, 2003). While less common, permanent leaf coloration also occurs and may be uniform or variegated across a leaf surface. Anthocyanins are the major class of plant pigments responsible for red, burgundy, and purple leaf coloration (Tanaka et al., 2008). Anthocyanins are secondary metabolites, synthesized through the flavonoid biosynthetic pathway, and are believed to be involved in a host of leaf

defenses, including herbivory, excess light (visible and ultraviolet), cold temperature stress, and nutrient deficiency (Chalker-Scott, 1999; Manetas, 2006).

Increases in anthocyanin content have been observed after exposure to high irradiance levels (Gong et al., 1997; Hughes et al., 2005; Islam et al., 2005; Kleinhenz et al., 2003), decreased temperature (Armitage and Carlson, 1982; Gazula et al., 2005; Oren-Shamir and Levi-Nissim, 1997b; Pietrini and Massacci, 1998), UV-B exposure (Krizek et al., 1998; Maekawa et al., 2001; Takahashi et al., 1991; Tsormpatsidis et al., 2008), and low media P levels (Dedaldechamp et al., 1995; Hodges and Nozzolillo, 1996; Yuan et al., 2009). In most studies, anthocyanin content is reported at one point in time, ranging from a couple days after a treatment to a few months (Hughes et al., 2005; Islam et al., 2005; Kim et al., 2006; Oren-Shamir and Levi-Nissim, 1997b; Piccaglia et al., 2002; Takahashi et al., 1991). It is not known how quickly differences in anthocyanin content can be observed after the start of treatments, how that may vary with species and cultivar, or how quickly leaf anthocyanin content can stabilize after environmental changes.

The introduction of plants with novel leaf coloration or increased pigmentation has been a goal of ornamental plant breeders in recent years. In addition, providing an ideal growing environment during the later stages of production to increase leaf pigmentation may encourage sales. Greenhouse production for spring markets occurs during winter and early spring, when irradiance in the northern US is low (Korczynski et al., 2002), which may not be ideal for anthocyanin accumulation. Also, irradiance levels in greenhouses are lower than outside, often by 40% to 60%, and transmission of UV

radiation is low (Wagner, 1998). For example, in a St. Paul, MN greenhouse, light transmission (400-700 nm) was reduced to 35% and UV-B transmission was 6% (Fig. 5.1). Our research objectives here were to 1) quantify changes in anthocyanin content in response to environmental factors (temperature, irradiance, UV-B radiation) and P availability, 2) determine how quickly differences in pigmentation occurred, and 3) determine which factor(s) had the greatest influence on altering pigment content. *Solenostemon scutellarioides* L. (Codd.) (coleus, Lamiaceae), *Panicum virgatum* L. (switchgrass, Poaceae), and *Pennisetum advena* Wipff & Veldkamp (purple fountaingrass; Poaceae) were selected for this study because they are common horticultural selections with attractive ornamental anthocyanic characteristics, represent two different classes of plants (C_3 and C_4), have different leaf morphologies, and have different cold tolerances (switchgrass is cold tolerant, coleus and purple fountaingrass are cold-intolerant).

Materials and Methods

Plant culture

Solenostemon scutellarioides (coleus) 'Versa Burgundy to Green', 'Versa Crimson Gold' and 'Versa Lime' seed (PanAmerican Seed, Ball Horticultural Company, West Chicago, IL) were sown (one seed per cell), in a 128-count cell tray filled with soilless media (Metro Mix 200; SunGro Horticulture, Bellvue, WA). Sown trays were placed under intermittent mist for 14 d, until seedlings had germinated and cotyledons

were horizontal to the media surface. Seedlings were then moved to a greenhouse and grown under ambient irradiance, plus 2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ lighting from incandescent bulbs from 2200-0200 $_{HR}$ (daily) to maintain plants in a vegetative state. Greenhouse air temperature set points were 22 °C day/18 °C night. Seedlings were transplanted 6 weeks after sowing into 10 cm diameter plastic square pots (volume = 815 cm^3) filled with LC-8 soilless media (SunGro Horticulture, Bellvue, WA), and the apical shoot was pruned to one node 10 d after transplant.

Coleus stock plants were maintained in 16.5 cm diameter pots (volume = 1.3 L) in a greenhouse (University of Minnesota, St. Paul, MN). Initial plants of 'Big Red Judy', 'Dipt in Wine', 'LifeLime', 'Royal Glissade' and 'Sedona' were received from Pleasant View Gardens (Loudon, NH); cuttings of 'Dark Star' and 'Solar Red' were harvested from the University of Minnesota trial and display garden (St. Paul, MN); and red and green-leaved selections of 'Twist and Twirl' were provided from the coleus breeding program at the University of Florida (courtesy of D.G. Clark). Greenhouse air temperature set points were 22 °C day/18 °C night, and plants received ambient irradiance plus night interruption lighting (2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from incandescent bulbs nightly from 2200-0200 $_{HR}$) to maintain a vegetative state. Actual air temperatures and mean daily light integrals (DLI) are reported in Table 5.1. Cuttings were pruned to two nodes, inserted into a 50-count cell tray (volume = 110 cm^3) filled with LC-8 soilless media and placed under intermittent mist (5 s every 15 min) for 7 d until root formation occurred. Plants were then moved into the greenhouse and transplanted 2 weeks later into 10 cm diameter square pots (volume = 815 cm^3) filled with LC-8 soilless media.

Plants were pruned to one node 10 d after transplant. Coleus were watered as needed and fertilized weekly with 14.3 mM N from a 20N-4.4P-16.6K fertilizer (Peter's 20-10-20; The Scott's Co., Marysville, OH). Experiments were initiated after 4 leaf pairs had unfolded on the axillary shoots (between 3 weeks and 5 weeks after the apical shoot was pruned). Actual air temperatures and mean daily light integrals (DLI) are reported in Table 5.1.

Panicum virgatum (switchgrass) 'Heavy Metal' and 'RR1' Ruby Ribbons™ (Emerald Coast Growers, Pensacola, FL) and *Pennisetum advena* (purple fountaingrass) (EuroAmerican Propagators, Bonsall, CA) stock plants were maintained in a greenhouse with ambient irradiance plus supplemental lighting from high-pressure sodium lamps (75 $\mu mol \cdot m^{-2} \cdot s^{-1}$) between 0600-2200 $_{HR}$ (16 h photoperiod) when ambient irradiance was less than 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (see temperature and irradiance data in Table 5.2). Each fall, switchgrass plants were moved into a cooler for 10 weeks of cold storage (2 °C to 4 °C, <10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ irradiance) to satisfy dormancy requirements. The timing of the cold treatment was staggered so actively-growing switchgrass plants would be available year-round. Following cold storage, plants were moved to the greenhouse and new shoots emerged 2 to 4 weeks later.

Switchgrass and purple fountaingrass plants were sectioned into divisions containing five to seven young tillers (< 5 cm tall) and mature, flowering stems, if present, were removed. Divisions were transplanted into 10 cm diameter square pots (volume = 815 cm^3) filled with SB500, a high-porosity soilless media (SunGro Horticulture, Bellvue, WA). Experiments began 6 to 8 weeks later. Plants were watered

as needed and fertilized weekly with 14.3 mM N from a 20N-4.4P-16.6K fertilizer (Peter's 20-10-20; The Scott's Co., Marysville, OH).

Experiments

Irradiance

Twelve coleus cultivars [nine anthocyanic ('Big Red Judy', 'Dark Star', 'Dipt in Wine', 'Royal Glissade', 'Sedona', 'Solar Red', 'Twist and Twirl' red, 'Versa Burgundy to Green', and 'Versa Crimson Gold') and three acyanic ('LifeLime', 'Twist and Twirl' green, and 'Versa Lime')], two switchgrass cultivars ['RR1' Ruby Ribbons^{TM} (red) and 'Heavy Metal' (green)], and purple fountaingrass were used in this experiment (Table 5.3). Plants were moved into growth chambers (1.4 m^2; Environmental Growth Chambers, Chagrin Falls, OH) maintained at 20 ± 1 °C leaf temperature, a 16 h photoperiod (0600-2200 $_{HR}$), and 50% relative humidity. Irradiance was 75, 150, 300, or 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation (*PAR*; DLI = 4.3, 8.6, 17.3, and 34.6 $mol \cdot m^{-2} \cdot d^{-1}$, respectively), provided with incandescent and fluorescent lamps. Leaf temperature and irradiance were checked weekly and adjusted as necessary. Plants were watered as needed and fertilized weekly with 14.3 mM N from a 20N-4.4P-16.6K fertilizer (Peter's 20-10-20; The Scott's Co., Marysville, OH). Every 4 d for 20 d, two leaf punches (0.3 cm^2) per plant were collected from a fully-expanded, non-shaded leaf, from the fourth leaf from the shoot apex for coleus and from the second leaf from the apex for grasses. One leaf sample was used to quantify relative anthocyanin content and the other to quantify chlorophyll content. At the end of the experiment (20 d), coleus leaf

area was calculated in ImageJ (U.S. Natl. Inst. Health, Bethseda, Maryland), using photographs of individual leaves, and the percentage of total leaves containing anthocyanins was determined for switchgrass and purple fountaingrass.

The experimental design was a randomized complete block design (three replicates in time). Day 0 for the three coleus replicates were 22 Jan., 9 Mar., and 20 Apr. 2010, and day 0 for the three switchgrass and purple fountaingrass replicates were 24 May, 7 June, and 21 June 2010. The layout was a split plot, with irradiance as the main plot, cultivar as the subplot, and repeated measures (day) on the subplot. In each replication, there were five plants per cultivar within each irradiance treatment (total n = 15 per treatment). Each species was analyzed separately for anthocyanin and chlorophyll content.

Temperature

From the 12 coleus cultivars in the irradiance experiment, a subset of five cultivars was selected for the temperature experiment [three anthocyanic ('Big Red Judy', 'Royal Glissade', and 'Twist and Twirl' red) and two acyanic ('LifeLime' and 'Twist and Twirl' green); Table 5.3] and they encompassed a range of anthocyanin pigmentation. Two switchgrass cultivars ['RR1' Ruby Ribbons^{TM} (red) and 'Heavy Metal' (green)] were used in this experiment. Purple fountaingrass was not used. Plants were moved into growth chambers (1.4 m^2; Environmental Growth Chambers, Chagrin Falls, OH) maintained at 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ irradiance (provided by fluorescent and incandescent lamps), a 16 h photoperiod (0600-2200 $_{HR}$), and 50% relative humidity. Air temperatures

were set to provide leaf temperatures of 12, 18, 24, or 30 °C. Leaf temperature and irradiance were checked weekly and adjusted as necessary. Plants were watered as needed and fertilized weekly with 14.3 mM N from a 20N-4.4P-16.6K fertilizer (Peter's 20-10-20; The Scott's Co., Marysville, OH). Every 5 d for 25 d, two leaf punches (0.3 cm^2) per plant were collected for anthocyanin and chlorophyll content (described in the irradiance experiment). The experimental design was a randomized complete block design (three replicates in time). Day 0 for the three coleus and switchgrass replicates were 6 Jan., 17 Feb., and 18 Mar. 2011. The layout was a split plot, with temperature as the main plot, cultivar as the subplot, andrepeated measures (day) on the subplot. In each replication, there were three plants per switchgrass cultivar and four plants per coleus cultivar in each temperature treatment (n = 9 and 12, respectively). Each species was analyzed separately.

Irradiance x temperature

Three coleus cultivars ('Big Red Judy', 'Royal Glissade', and 'LifeLime') and two switchgrass cultivars ('RR1' Ruby Ribbons™ and 'Heavy Metal') were used in this experiment. Plants were moved into six growth chambers (1.4 m^2; Environmental Growth Chambers, Chagrin Falls, OH) set to provide a factorial combination of three leaf temperatures (12, 18, or 24 °C) and two irradiances (200 or 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Light was provided by fluorescent and incandescent lamps (16 h photoperiod, 0600-2200 $_{HR}$) and relative humidity was 50%. Leaf temperature and irradiance were checked weekly and adjusted as necessary. Plants were watered as needed and fertilized weekly with 14.3

mM N from a 20N-4.4P-16.6K fertilizer (Peter's 20-10-20; The Scott's Co., Marysville, OH). Every 5 d for 25 d, two leaf punches (0.3 cm^2) per plant were collected for anthocyanin and chlorophyll content (described in the irradiance experiment). The experimental design was a randomized complete block design (three replicates in time). Day 0 for the three coleus replicates were 19 Dec. 2011, 4 Jan. 2012, and 24 Jan. 2012, and day 0 for the switchgrass replicates were 4 Apr., 26 Apr., and 16 May 2012. The layout was a split plot, with the temperature/irradiance treatment as the main plot, cultivar as the subplot, and repeated measures (day) on the subplot. In each replication, there were three switchgrass plants per cultivar and four coleus plants per cultivar in each treatment (n = 9 and 12, respectively). Each species was analyzed separately.

Supplemental UV-B radiation

Eight coleus cultivars ('Big Red Judy', 'Dark Star', 'Dipt in Wine', 'LifeLime', 'Royal Glissade', 'Sedona', 'Twist and Twirl' green, and 'Twist and Twirl' red) and two switchgrass cultivars ('RR1' Ruby Ribbons™ and 'Heavy Metal') were used in this experiment (Apr. 2012 for coleus and June 2012 for switchgrass). Plants were moved into a growth chamber (1.4 m^2; Environmental Growth Chambers, Chagrin Falls, OH) maintained at 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ irradiance (provided by fluorescent and incandescent lamps), a 16 h photoperiod (0600-2200 $_{HR}$), 20 °C leaf temperature, and 50% relative humidity. UV-B radiation (280-320 nm) was 3 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (280-320 nm). Plants were watered as needed and fertilized weekly with 14.3 mM N from a 20N-4.4P-16.6K fertilizer (Peter's 20-10-20; The Scott's Co., Marysville, OH). After a 7 d acclimation

period, plants were moved to another chamber and exposed to supplemental UV-B radiation (13 µmol·m^{-2}·s^{-1} supplemental, 16 µmol·m^{-2}·s^{-1} total) provided by two lamps (peak at 313 nm, Philips UV-B TL 100 W/01). Following exposure durations of 0, 1, 2, 4, 8, 24, or 48 h, plants were returned to ambient conditions. Three days later, two leaf punches (0.3 cm^2) per plant were collected for anthocyanin and chlorophyll content (described in the irradiance experiment). The experimental design was a randomized complete block design (n=3), and each species was analyzed separately.

Phosphorus

Three coleus cultivars ('Big Red Judy', 'Royal Glissade', and 'Twist and Twirl' red) and two acyanic ('LifeLime' and 'Twist and Twirl' green)] and two switchgrass cultivars ['RR1' Ruby Ribbons^{TM} (red) and 'Heavy Metal' (green)] were used in this experiment. Plants were irrigated with water only (no fertilizer) for 1 week before the start of treatments to reduce the residual nutrient pool in the media. They were moved into a growth chamber (3.3 m^2; Environmental Growth Chambers, Chagrin Falls, OH) maintained at 300 µmol·m^{-2}·s^{-1} irradiance (provided by fluorescent and incandescent lamps), a 16 h photoperiod (0600-2200 $_{HR}$), 20 °C leaf temperature, and 50% relative humidity. Leaf temperature and irradiance were checked weekly and adjusted as necessary. Plants were fertilized at every watering interval (approximately every other day) with 14.3 mM N and 4.3 mM K (The Scott's Co., Marysville, OH), and P rates were adjusted to 0 (deionized water), 0.5, or 1 mM. A fourth treatment was irrigated solely with deionized water (no fertilizer). Once a week for 4 weeks, two leaf punches (0.3

cm^2) per plant were collected for anthocyanin and chlorophyll content (as described in the irradiance experiment). The experimental design was a randomized complete block design (two replicates in time, Oct. and Nov. 2012), with repeated measures (week). In each replication, there were two plants per switchgrass cultivar and three plants per coleus cultivar in each treatment (n = 4 and 6, respectively). Each species was analyzed separately.

Pigment quantification

Two leaf discs, one for anthocyanin and one for chlorophyll content, were sampled from one mature leaf per plant at each sampling interval. Each leaf disc (0.3 cm^2) was placed in a 1.7 mL microcentrifuge tube containing either 1 mL of 99:1 (v:v) methanol:HCl (for anthocyanin extraction) or 95% ethanol (for chlorophyll extraction). Two tungsten carbide beads were added to each tube containing a switchgrass leaf disc. Switchgrass leaf tissue was homogenized with a Mixer Mill (MM 300; Qiagen, Valencia, CA) for 4 min., then placed in the dark at 4 °C for 18 h. Coleus leaf discs were transferred directly into cold storage without homogenization of the leaf tissue. Following cold storage, switchgrass samples were centrifuged (model 5714R; Eppendorf, Hamburg, Germany) at 4 °C for 10 min at 10,000 g. A 300 µL aliquot from each microcentrifuge tube was pipeted into a 96-well plate and absorbance was measured using a plate reader with monochromator optics (SpectraMax 190; Molecular Devices, Sunnyvale, CA). Maximum absorbance for anthocyanin was 530 nm for all species and cultivars. Chlorophyll was measured at 649 and 665 nm. Absorbance values were

normalized to a 1 cm pathlength and a 1 cm^2 leaf area. Anthocyanin content was calculated as cyanidin-3-glucoside (Siegelman and Hendricks, 1958), and chlorophyll was calculated using equations published by Wintermans and De Mots (1965).

Statistical analysis

Data were analyzed in SAS (SAS 9.3, Cary, NC), and mean separation was conducted for sources of variation significant at $P<0.05$ using Tukey's HSD ($\alpha=0.05$). PROC MIXED was used for experiments with split plot layouts and PROC GLM was used for experiments without split plot layouts. For experiments with repeated measures, a REPEATED statement was included.

Results

Irradiance

In all three species (coleus, switchgrass, and purple fountaingrass), anthocyanin content increased in red-leaved cultivars as irradiance increased. After 20 d, anthocyanin content increased at each irradiance in five of 12 coleus cultivars ('Big Red Judy', 'Dark Star', 'Dipt in Wine', 'Royal Glissade', and 'Twist and Twirl' red; Table 5.4). In two cultivars ('Sedona' and 'Versa Crimson Gold'), anthocyanin content was lower in leaves exposed to 75 $\mu mol \cdot m^{-2} \cdot s^{-1}$ than in leaves exposed to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, but not 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$. At the highest irradiance, loss of pigmentation occurred as a result of excess irradiance (Fig. 5.2). Anthocyanin content barely changed in the three green-

leaved coleus cultivars (‘LifeLime, ‘Twist and Twirl’ green, and ‘Versa Lime’) (Table 5.4).

In purple fountaingrass, anthocyanin content was lowest in leaves exposed to 75 $\mu mol \cdot m^{-2} \cdot s^{-1}$, intermediate in those exposed to 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and highest in leaves exposed to 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 5.3A). Red switchgrass (Ruby Ribbons^{TM}) leaves accumulated the greatest anthocyanin content in leaves exposed to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ or 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Anthocyanin content was lower at 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and lowest at 75 $\mu mol \cdot m^{-2} \cdot s^{-1}$(Fig. 5.3B). In green switchgrass (‘Heavy Metal’), anthocyanin content was lower than in Ruby Ribbons^{TM} at all irradiances. Very little anthocyanin accumulated in plants exposed to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ or less (7.1 to 7.4 $\mu g \cdot cm^{-2}$, pooled across all days), but averaged 8.5 $\mu g \cdot cm^{-2}$ in plants grown under 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 5.3C).

Coleus leaves have constitutive foliar anthocyanin pigmentation at all leaf developmental stages. In switchgrass and purple fountaingrass, some leaves are red and some are green. Environmental conditions may alter this ratio and in this study, the percent of total leaves exhibiting partial or complete red leaf coloration increased as irradiance increased (Fig. 5.4). After 20 d, 18% of purple fountaingrass leaves were red when plants were grown at 75 $\mu mol \cdot m^{-2} \cdot s^{-1}$ but increased to 100% at 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$. In switchgrass Ruby Ribbons^{TM}, it increased from 10% at 75 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 83% at 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$. None of the leaves of switchgrass ‘Heavy Metal’ exhibited leaf reddening at or below 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, but 17% of leaves were red on plants exposed to 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Table 5.5).

In most coleus cultivars, differences in anthocyanin content between irradiances appeared after 8 d. For example, differences in anthocyanin content between lowest and highest irradiance (75 and 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$) treatments occurred after 4 d in two cultivars and after 8 d in 6 cultivars (Fig. 5.5). Differences in responses between 75 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ treatments occurred after 8 d in eight of nine red-leaved cultivars.

In red switchgrass, differences in anthocyanin content between 75 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 300 or 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ treatments occurred after 8 d, with differences between 75 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ treatments occurring after 20 d (Fig. 5.3B). In purple fountaingrass, anthocyanin content in leaves exposed to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ or less was lower than in leaves exposed to 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ after just 4 d (Fig. 5.3A), and differences in anthocyanin content between 75 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 150 or 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ treatments occurred at the subsequent sampling interval (8 d). Overall, changes in anthocyanin content as a result of increased irradiance could be observed after approximately 1 week in both coleus and grasses.

The length of time necessary to achieve maximum anthocyanin content varied with irradiance and species. At 75 $\mu mol \cdot m^{-2} \cdot s^{-1}$, five of nine red coleus cultivars had no change in anthocyanin content across the duration of the experiment, and the other cultivars reached maximum concentrations between 4 d and 16 d (Fig. 5.5). At 150, 300, and 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$, anthocyanin content was still increasing after 20 d in three cultivars (‘Big Red Judy’, ‘Dipt in Wine’, and ‘Royal Glissade’; Fig. 5.5). From an aesthetic standpoint, anthocyanin content in these three cultivars was acceptable at or

before 20 d, and the continued increase in anthocyanin content did not further enhance marketability. In red switchgrass and purple fountaingrass, anthocyanin content at 75 $\mu mol \cdot m^{-2} \cdot s^{-1}$ did not change across the duration of the experiment. Maximum anthocyanin content at 150, 300, and 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ occurred after 8, 8, and 20 d, respectively, in Ruby Ribbons™ switchgrass and purple fountaingrass (Fig. 5.3).

Chlorophyll content decreased as irradiance increased in all species. At 20 d, differences in chlorophyll content between irradiance treatments occurred in eight of 12 coleus cultivars (Table 5.6), and in both grass species (data not shown). As a result, the anthocyanin/chlorophyll ratio increased and may have resulted in even greater visual differences between leaves grown at low and high irradiances. However, plant growth at higher irradiances did not suffer as a result of the decreased chlorophyll content (personal observation).

Temperature

Coleus cultivars responded to temperature, but switchgrass did not ($P>0.05$). Two of three red coleus cultivars ('Big Red Judy' and 'Twist and Twirl' red) accumulated more anthocyanin at lower temperatures than at higher temperatures, while 'Royal Glissade' accumulated less anthocyanin at lower temperatures (Fig. 5.6 and 5.7). After 25 d, anthocyanin content in 'Big Red Judy' was greatest in plants exposed to 12 °C and similar among plants grown at 18, 24, and 30 °C (Table 5.7). Anthocyanin content in 'Twist and Twirl' red was similar at 12 °C and 18 °C but greater than in plants exposed to 24 °C or 30 °C. 'Royal Glissade' leaves accumulated less anthocyanin at 12

°C than at 18 °C or 30 °C. Anthocyanin content only showed minor variation in green-leaved cultivars across all temperatures.

Differences in anthocyanin content between temperature treatments occurred after a minimum of 15 d in responsive coleus cultivars (Fig. 5.6A-C). After 15 d, 'Big Red Judy' and 'Twist and Twirl' red had a higher anthocyanin content at 12 °C than at higher temperatures, and after 20 d, 'Twist and Twirl' red plants exposed to 12 °C or 18 °C had more anthocyanin than plants grown at 24 °C or 30 °C.

Total chlorophyll content during the exposure duration decreased in plants exposed to 12 °C, remained constant at 18 °C and 24 °C, and increased at 30 °C (Fig. 5.6D).

Interaction of irradiance and temperature

Since both temperature and irradiance influenced anthocyanin content in coleus, we investigated the possibility of an interaction between the two factors. After 25 d, there was an interaction between cultivar and temperature and between cultivar and irradiance ($P<0.001$ for both). Consistent with our previous experiments, anthocyanin content in red-leaved coleus ('Big Red Judy' and 'Royal Glissade') was greater at 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ than at 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and similar in 'LifeLime' at both irradiances (Table 5.8). In response to temperature, 'Big Red Judy' had greater anthocyanin content at 12 °C relative to 18 °C or 24 °C, but 'Royal Glissade' had increased anthocyanin content as temperature increased (Table 5.8).

No differences in anthocyanin content between temperature/irradiance treatments were detected until 20 d in 'Big Red Judy' (Fig. 5.8A). The combination of low temperature and high irradiance (12 °C/400 μmol·m^{-2}·s^{-1}) resulted in a higher anthocyanin content than either 18 °C or 24 °C at 200 μmol·m^{-2}·s^{-1}. In 'Royal Glissade', differences in anthocyanin content between treatments started as early as 5 d, in which 24 °C/400 μmol·m^{-2}·s^{-1} resulted in greater anthocyanin content than at 12 °C/400 μmol·m^{-2}·s^{-1} (Fig. 5.8B). By 25 d, plants exposed to 18 °C/400 μmol·m^{-2}·s^{-1} or 24 °C (200 and 400 μmol·m^{-2}·s^{-1}) had more anthocyanin content than those grown at 12 °C (200 or 400 μmol·m^{-2}·s^{-1}). As a result of exposure to 12 °C, 'Royal Glissade' leaves began to turn pink due to the concurrent loss of anthocyanin and chlorophyll.

In switchgrass, there was no interaction between temperature and irradiance. Irradiance was significant, which concurred with our previous studies. Anthocyanin content at 200 μmol·m^{-2}·s^{-1} was less than at 400 μmol·m^{-2}·s^{-1} (17.9 μg·cm^{-2} and 23.3 μg·cm^{-2}, respectively, pooled across cultivar and temperature).

UV-B

Exposure to supplemental UV-B radiation for up to 48 h did not change anthocyanin content in seven of eight coleus cultivars or either switchgrass cultivar. Coleus 'Dark Star' was the only cultivar to respond with increased anthocyanin content after an 8 h exposure, with an additional increase after 24 h exposure (data not shown).

Phosphorus

Reduced phosphorus application, in conjunction with standard applications of N and K, did not impact anthocyanin content in coleus or switchgrass leaves at any sampling interval (from 0 to 4 weeks). Additionally, watering with deionized water only was also ineffective at altering anthocyanin content in either species.

Discussion

Temperature and irradiance were the major factors influencing anthocyanin accumulation in red coleus cultivars examined, whereas irradiance was the major factor influencing anthocyanin content in switchgrass and purple fountaingrass. Decreased application rates of P or short-term supplemental UV-B radiation did not influence anthocyanin content in either coleus or switchgrass (with the exception of coleus 'Dark Star', which had increased leaf anthocyanin content in response to supplemental UV-B exposure).

Anthocyanins in coleus leaves and stems were localized in the upper and lower epidermis and trichomes (Fig. 5.9A). In switchgrass, anthocyanins also localized in the upper epidermis (Fig. 5.9B) but accumulated in the lower epidermis as well if leaves were exposed to high irradiance or were inverted so that the abaxial surface was illuminated (personal observation). Anthocyanin accumulation was a cell-specific response in some taxa. In coleus 'Royal Glissade' and switchgrass Ruby Ribbons^{TM}, anthocyanic epidermal cells were sometimes located adjacent to non-anthocyanic cells (Fig. 5.9C).

If anthocyanin content increased, it accumulated in cell layers already producing anthocyanins (i.e. epidermal cells) and did not accumulate in mesophyll cells. In the two taxa that had both red and green epidermal cells under greenhouse conditions (coleus 'Royal Glissade' and switchgrass Ruby Ribbons^{TM}), the ratio of red to green epidermal cells increased in response to favorable environmental stimuli. Therefore, the observed increase in anthocyanin content likely resulted from both an increase in anthocyanin content in cells that were anthocyanic at the start of the experiment as well as an increase in the total number of epidermal cells containing anthocyanins.

Anthocyanin content in all red coleus cultivars increased in response to increased irradiance, but the magnitude of the response was cultivar specific. For example, anthocyanin content increased between 58% and 387% in red coleus leaves exposed to 75 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 20 d ('Dipt in Wine' and 'Big Red Judy', respectively). At 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the increase in anthocyanin content ranged between 267% and 2031% ('Versa Burgundy to Green' and 'Royal Glissade', respectively). Cultivars with the greatest increase in anthocyanin content at 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were 'Big Red Judy', 'Royal Glissade', and 'Twist and Twirl' red (all > 700% increase, data not shown). All of these cultivars are selections from the University of Florida coleus breeding program and have excellent foliage coloration under full sun conditions (D.G. Clark, personal communication).

Leaf area in coleus decreased as irradiance increased (data not shown), which might have resulted in a concentrating effect of the anthocyanins when sampled. Using leaf area measurements, we calculated total anthocyanin content per leaf and observed

that anthocyanin content increased in all red-leaved cultivars on both a per unit area ($\mu g \cdot cm^{-2}$) and per leaf ($\mu g \cdot leaf^{-1}$) basis as irradiance increased.

In some coleus cultivars ('Sedona', 'Solar Red', 'Versa Burgundy to Green', and 'Versa Crimson Gold'), exposure to 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ resulted in increased anthocyanin content initially, but a loss of pigmentation (photobleaching) started to appear after 12 d to 16 d (Fig. 5.2). This occurred on leaves exposed to direct light but not on leaves shaded from direct exposure. This has also been reported in *Perilla frutescens* cell suspension cultures (Zhong et al., 1991), in which anthocyanin content increased as irradiance increased, up to 27.2 $W \cdot m^{-2}$, then decreased at 54.4 $W \cdot m^{-2}$. In our study, optimal irradiance for increased anthocyanin content was 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for some coleus cultivars (a fluence rate that did not result in leaf damage due to excess irradiance), and 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the other coleus cultivars. This agrees with their designations as "partial shade" or "full sun" cultivars.

Differences in anthocyanin content in response to irradiance have been reported after as little as 7 d in *Ipomoea batatas* (Islam et al., 2005), which was similar to the length of time needed to observe difference in our species. The response time may be as long as 2-3 months, however, as reported for *Galax urceolata* (Hughes et al., 2005), but most studies reported changes in anthocyanin content after 3-4 weeks (Armitage and Carlson, 1982; Gong et al., 1997; Kleinhenz et al., 2003; Richards et al., 2004). However, data was typically reported for one time point, so it is not known if differences in anthocyanin content occurred prior to or after the reported duration length.

In coleus, purple fountaingrass, and red switchgrass, we observed that the initial response was relatively quick, and incremental increases in anthocyanin content continued for a period of time after that. The length of time necessary to achieve maximum anthocyanin content at high light intensities was cultivar-specific, ranging between 8 d and >20 d at 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the three species evaluated (Fig. 5.3 and 5.5). Red switchgrass and purple fountaingrass should be grown at high light intensities to increase overall leaf and canopy redness and 'Heavy Metal' should not be grown at high light intensities if leaf reddening during propagation and production is undesirable.

Temperature influenced anthocyanin content in coleus but not switchgrass, and the response was cultivar specific. Anthocyanin content in 'Big Red Judy' and 'Twist and Twirl' red was greatest at the lowest temperature examined (12 °C), whereas anthocyanin content in 'Royal Glissade' was lowest at 12 °C. While differential responses in anthocyanin content to temperature have been observed in different species, it has not been reported for cultivars within a species. Increased foliar anthocyanin content at lower temperatures is a more common response and has been reported for *Arabidopsis thaliana* (Hasdai et al., 2006), *Cotinus coggygria* (Oren-Shamir and Levi-Nissim, 1997b), *Ipomoea batatas* (Islam et al., 2005), *Lactuca sativa* (Gazula et al., 2005), *Tagetes patula* (Armitage and Carlson, 1982) and *Zea mays* (Christie et al., 1994; Kim et al., 2006; Pietrini and Massacci, 1998). However, total anthocyanin production of *Fragaria ananassa* cell suspension cultures was greater at moderate temperatures than at low temperatures (Zhang et al., 1997).

Although anthocyanin content was greatest in coleus ‘Big Red Judy’ and ‘Twist and Twirl’ red at 12 °C, this temperature would not be recommended for production due to the adverse effect on plant growth and development observed for all cultivars. Observed effects included decreased chlorophyll content, reduced leaf size, slower growth rate, and partial leaf necrosis (green-leaved cultivars only). However, ‘Twist and Twirl’ red could be grown at 18 °C and have both enhanced anthocyanin content (similar to 12 °C) and acceptable plant quality. ‘Big Red Judy’ could be grown at temperatures ranging from 18 °C to 30 °C without any effect on anthocyanin content, although it would be lower than at 12 °C.

The possibility of an interaction between irradiance and temperature was examined in coleus and switchgrass. As a precedent, Boo et al., (1997) observed in *Cichorium intybus* that anthocyanin content increased with irradiance in plants exposed to low temperature but was unaffected by irradiance at higher temperatures. However, there was no interaction between temperature, irradiance, and cultivar in switchgrass, and it appears that irradiance is the primary environmental influence of anthocyanin content. In coleus, responses to temperature and light were similar to those observed individually in the single factor studies.

Although increased foliar pigmentation has been observed in response to supplemental UV-B radiation or decreased P application rates in other species, neither influenced anthocyanin content in coleus or switchgrass. The supplemental UV-B fluence rate chosen (13 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was similar to that used previously on coleus (12 $\mu mol \cdot m^{-2} \cdot s^{-1}$; Burger and Edwards, 1996). Increased anthocyanin content in response to

UV-B has been observed within 48 h (Snell at al., 2009; Takahashi et al., 1991), although experiments have reported anthocyanin content after as long as eight weeks of treatments (Boo et al., 1997). No change in anthocyanin content was detected in switchgrass plants exposed to supplemental UV-B (16 h per day) for durations as long as 2 weeks (data not shown).

Researchers have detected differences in anthocyanin content in response to P application rates in less than 1 week in grape cell suspension cultures (Dedaldechamp et al., 1995) and in *Zea mays* field experiments (Cobbina and Miller, 1987), so duration may or may not have been long enough to observe detectable differences in anthocyanin content. The lack of an anthocyanin accumulation response to low P has been observed in *Tagetes patula* (Armitage and Carlson, 1982), *Brassica oleracea* (Piccaglia et al., 2002), coleus (Henry et al., 2012), and cauliflower, kohlrabi, and radish (Hodges and Nozzolillo, 1996). One possible explanation for a lack of observed differences in anthocyanin due to low P might be that any change in anthocyanin content as a result of decreased P application may have been too small to be detected in leaves with constitutive anthocyanin pigmentation and was masked by the underlying baseline level of anthocyanins present.

Increases in anthocyanin content may be due to increased anthocyanin biosynthesis, increased stability, or decreased degradation. Anthocyanin biosynthesis is regulated by transcription factors, which can be regulated by light and/or cold temperature (Chalker-Scott, 1999; Steyn et al., 2002), and anthocyanin degradation may occur through the activity of peroxidases or oxidases (Oren-Shamir, 2009). In *Brunsfelia*

calycina, changes in flower color from purple to white resulted from active degradation due to increased peroxidase activity (Vaknin et al., 2005), but leaf greening in *Arabidopsis thaliana* plants overexpressing the transcription factor *PAP1*, which leads to increased anthocyanin synthesis, upon transfer from room temperature to a high temperature/low light environment was not the result of increased degradation by peroxidases or oxidases (Rowan et al., 2009). In our studies, we quantified relative anthocyanin content but not changes in biosynthesis or degradation and it is plausible that both may have contributed to our observed differences in anthocyanin content in response to temperature and irradiance.

Anthocyanin degradation is greater at higher temperature in juice extracts (Attoe and von Elbe, 1981) and in fruits (Steyn et al., 2005), and mRNA of anthocyanin biosynthesis genes in grape skins have been shown to decrease in response to elevated temperatures (Yamane et al., 2006). This combination of increased anthocyanin degradation and reduced anthocyanin biosynthesis is a plausible hypothesis for the observed decrease in anthocyanin content at higher temperatures in coleus 'Big Red Judy' and 'Twist and Twirl' red, but it does not explain why 'Royal Glissade' had increased anthocyanin content at higher temperatures. Anthocyanin transcription factors are light induced (Mancinelli, 1983) and the increased anthocyanin content observed at higher irradiances may be due to increased anthocyanin biosynthesis and minimal changes in anthocyanin degradation (air temperatures were adjusted to compensate for the greater heat output from lamps at the higher irradiances so that leaf temperatures were very similar in all irradiance levels).

Foliar anthocyanin content can be influenced by a myriad of factors, including temperature, irradiance, UV-B radiation, nutrient status, carbohydrate status, exogenously applied plant growth regulators, and/or salinity (Chalker-Scott, 1999; Close and Beadle, 2003; Manetas, 2006; Steyn et al., 2002). In our studies, irradiance strongly influenced anthocyanin content in switchgrass and purple fountaingrass. Temperature and irradiance, but not UV-B or low P, primarily increased anthocyanin content in red coleus. The responses were cultivar specific, however, especially in response to temperature. To help improve red foliar pigmentation for improved aesthetic appeal at the retail level, growers can adjust the greenhouse environment of these species during the last 1 to 2 weeks of production to increase anthocyanin content without incurring unnecessary production expenses.

Table 5.1. Average greenhouse daily light integral (DLI, $mol \cdot m^{-2} \cdot d^{-1}$) and temperature (day and night, °C) conditions during *Solenostemon scutellarioides* (coleus) stock plant maintenance, propagation, and production (means ± SD) for each experiment.

Experiment	Growing condition	Rep	DLI ($mol \cdot m^{-2} \cdot d^{-1}$)	Day temperature (°C)	Night temperature (°C)
Irradiance	Stock plants	1	7.6 ± 1.7	22.0 ± 0.9	17.0 ± 0.1
		2	6.1 ± 1.4	23.4 ± 1.5	18.0 ± 0.2
		3	6.4 ± 2.9	22.6 ± 1.4	17.8 ± 0.3
	Propagation	1	6.9 ± 0.7	21.8 ± 1.0	17.1 ± 0.1
		2	5.0 ± 2.5	22.0 ± 1.2	17.7 ± 0.2
		3	11.6 ± 5.1	22.6 ± 2.2	18.5 ± 0.5
	Production	1	5.7 ± 1.5	23.1 ± 1.5	17.9 ± 0.2
		2	10.3 ± 4.5	23.1 ± 1.7	18.2 ± 0.5
		3	15.6 ± 6.2	23.4 ± 2.2	18.6 ± 0.8
Temperature	Stock plants	1	11.3 ± 4.4	25.2 ± 2.9	18.8 ± 1.9
		2	8.7 ± 4.8	23.6 ± 1.2	19.1 ± 1.7
		3	10.2 ± 1.2	21.8 ± 0.9	17.2 ± 0.5
	Propagation	1	24.7 ± 1.3	17.9 ± 0.4	17.8 ± 3.0
		2	21.9 ± 0.9	17.1 ± 0.5	17.7 ± 1.9
		3	21.7 ± 0.6	17.4 ± 0.3	17.6 ± 1.4
	Production	1	22.3 ± 1.1	17.7 ± 0.4	17.0 ± 3.8
		2	21.9 ± 0.7	17.3 ± 0.5	17.3 ± 2.0
		3	22.6 ± 0.7	16.9 ± 0.3	16.2 ± 2.8
Temp x Irradiance	Stock plants	1	8.2 ± 3.0	22.9 ± 2.3	19.7 ± 1.9
		2	12.3 ± 4.2	21.7 ± 1.8	17.6 ± 1.3
		3	15.3 ± 1.2	22.5 ± 0.6	17.2 ± 0.4
	Propagation	1	13.1 ± 4.3	21.5 ± 2.3	17.3 ± 1.7
		2	14.9 ± 1.2	22.4 ± 0.7	17.2 ± 0.4
		3	13.9 ± 1.1	22.2 ± 0.5	16.7 ± 0.1
	Production	1	14.8 ± 1.3	22.4 ± 0.6	17.2 ± 0.4
		2	14.1 ± 1.0	22.2 ± 0.5	17.0 ± 0.4
		3	13.8 ± 1.2	22.2 ± 0.5	16.7 ± 0.1

Table 5.1 (cont.).

Experiment	Growing condition	Rep	DLI ($mol \cdot m^{-2} \cdot d^{-1}$)	Day temperature (°C)	Night temperature (°C)
Phosphorus	Stock plants	1	14.6 ± 2.0	27.3 ± 1.8	12.7 ± 1.9
		2	14.2 ± 2.3	26.4 ± 2.5	17.9 ± 2.6
	Propagation	1	11.8 ± 2.0	22.9 ± 2.0	17.8 ± 2.1
		2	11.6 ± 1.4	22.5 ± 1.4	17.9 ± 1.1
	Production	1	8.4 ± 3.1	22.4 ± 2.6	17.4 ± 2.2
		2	9.8 ± 4.5	22.5 ± 2.4	17.0 ± 2.3
UV	Stock plants	1-3	12.5 ± 1.9	21.8 ± 0.7	17.3 ± 0.3
	Propagation	1-3	15.1 ± 1.8	22.4 ± 0.8	16.9 ± 0.2
	Production	1-3	19.7 ± 4.7	23.0 ± 0.7	16.8 ± 0.3

Table 5.2. Average greenhouse daily light integral (DLI, $mol \cdot m^{-2} \cdot d^{-1}$) and temperature (day and night, °C) conditions during *Panicum virgatum* (switchgrass) and *Pennisetum advena* (purple fountaingrass) propagation and production (means ± $_{SD}$) for each experiment.

Experiment	Growing condition	Rep	DLI ($mol \cdot m^{-2} \cdot d^{-1}$)	Day temperature (°C)	Night temperature (°C)
Irradiance	Propagation	1	9.1 ± 3.1	23.2 ± 1.5	18.1 ± 0.4
		2	14.1 ± 6.3	22.4 ± 2.2	18.6 ± 0.4
		3	16.7 ± 6.1	24.1 ± 2.3	18.7 ± 1.1
	Production	1	19.8 ± 4.8	22.6 ± 1.5	16.9 ± 0.4
		2	19.6 ± 6.1	23.5 ± 3.9	18.9 ± 2.4
		3	17.6 ± 6.1	23.7 ± 3.6	19.8 ± 2.6
Temperature	Propagation	1	24.7 ± 1.3	17.9 ± 0.4	17.8 ± 3.0
		2	21.9 ± 0.9	17.1 ± 0.5	17.7 ± 1.9
		3	21.7 ± 0.6	17.4 ± 0.3	17.6 ± 1.4
	Production	1	22.3 ± 1.1	17.7 ± 0.4	17.0 ± 3.8
		2	21.9 ± 0.7	17.3 ± 0.5	17.3 ± 2.0
		3	22.6 ± 0.7	16.9 ± 0.3	16.2 ± 2.8
Temp x Irradiance	Propagation	1	14.5 ± 1.9	22.3 ± 0.7	17.1 ± 0.3
		2	19.8 ± 4.6	23.0 ± 0.7	16.7 ± 0.3
		3	21.5 ± 5.4	23.2 ± 0.8	17.8 ± 0.9
	Production	1	19.8 ± 4.6	23.0 ± 0.7	16.7 ± 0.3
		2	21.6 ± 5.0	23.8 ± 0.3	16.8 ± 0.3
		3	18.5 ± 6.4	21.0 ± 2.1	17.8 ± 0.9
Phosphorus	Propagation	1	11.8 ± 2.0	22.9 ± 2.0	17.8 ± 2.1
		2	11.6 ± 1.4	22.5 ± 1.4	17.9 ± 1.1
	Production	1	8.4 ± 3.1	22.4 ± 2.5	17.4 ± 2.2
		2	9.8 ± 4.5	22.5 ± 2.4	17.0 ± 2.3
UV	Propagation	1	14.5 ± 1.9	22.3 ± 0.7	17.1 ± 0.3
		2	21.2 ± 5.6	22.7 ± 2.5	17.1 ± 0.6
	Production	1	20.0 ± 4.6	23.1 ± 0.7	16.7 ± 0.3
		2	17.1 ± 5.9	23.8 ± 3.5	19.9 ± 2.5

Table 5.3. Plant species and cultivars used in each experiment (irradiance, temperature, temperature x irradiance, supplemental UV-B, and P application rate).

Species	Cultivar	Leaf color	Experiment				
			Irradiance	Temperature	Temperature x Irradiance	UV-B	Phosphorus
Solenostemon scutellarioides	'Big Red Judy'	red	x	x	x	x	x
	'Dark Star'	purple	x			x	
	'Dipt in Wine'	dark red	x			x	
	'LifeLime'	green	x	x	x	x	x
	'Royal Glissade'	red/green	x	x	x	x	x
	'Sedona'	orange	x			x	
	'Solar Red'	red	x				
	'Twist and Twirl' green	green	x	x		x	
	'Twist and Twirl' red	red	x	x		x	
	'Versa Burgundy to Green'	dark red	x				
	'Versa Crimson Gold'	red	x				
	'Versa Lime'	green	x				
Panicum virgatum	'Heavy Metal'	green	x	x	x	x	x
	Ruby Ribbons™	red	x	x	x	x	x
Pennisetum advena	-	red	x				

Table 5.4. Anthocyanin content (μg·cm^{-2}) of *Solenostemon scutellarioides* (coleus), *Panicum virgatum* (switchgrass), and *Pennisetum advena* (purple fountaingrass) in response to 20 d at four irradiances (mean ± $_{SE}$, n=15). For each taxa, means within each row followed by different uppercase letters and means down each column followed by different lowercase letters are significantly different using Tukey's HSD (α = 0.05).

Taxa	Cultivar	Irradiance (μmol·m^{-2}·s^{-1})			
		75	150	300	600
Solenostemon scutellarioides	'Dark Star'	66.9 ± 3.9 Da	96.3 ± 5.5 Ca	126.3 ± 6.2 Ba	163.1 ± 10.1 Aa
	'Solar Red'	38.2 ± 2.6 Cb	46.4 ± 3.8 BCb	60.6 ± 3.0 Ac	55.5 ± 2.0 ABd
	'Twist and Twirl' Red	34.0 ± 2.3 Dbc	49.9 ± 2.5 Cb	63.9 ± 3.7 Bc	76.6 ± 3.8 Ac
	'Big Red Judy'	30.4 ± 1.7 Dbc	45.2 ± 3.9 Cb	58.4 ± 4.0 Bcd	74.0 ± 5.8 Ac
	'Dipt in Wine'	30.0 ± 3.1 Dbc	51.0 ± 4.5 Cb	77.3 ± 7.2 Bb	87.6 ± 7.4 Ab
	'Sedona'	26.5 ± 1.6 Bcd	32.4 ± 1.1 ABc	38.9 ± 2.6 Af	30.7 ± 3.6 ABe
	'Versa Crimson Gold'	23.5 ± 2.8 Bcd	33.6 ± 2.5 ABc	43.8 ± 2.7 Af	31.4 ± 7.4 Be
	'Versa Burgundy to Green'	22.9 ± 2.2 Ccd	32.9 ± 5.4 BCc	48.2 ± 4.5 Aef	39.8 ± 6.7 ABc
	'Royal Glissade'	17.1 ± 2.3 Dd	33.8 ± 3.4 Cc	51.1 ± 2.0 Bde	65.2 ± 2.8 Ac
	'Twist and Twirl' Green	3.4 ± 0.5 Ae	3.8 ± 0.3 Ad	3.1 ± 0.4 Ag	5.1 ± 0.7 Af
	'Versa Lime'	2.1 ± 0.2 Ae	1.8 ± 0.1 Ad	1.9 ± 0.2 Ag	2.0 ± 0.1 Af
	'LifeLime'	1.9 ± 0.1 Ae	2.0 ± 0.2 Ad	1.6 ± 0.1 Ag	2.0 ± 0.2 Af
Panicum virgatum	'Heavy Metal'	7.4 ± 0.4 Aa	6.3 ± 0.3 Ab	6.0 ± 0.3 Ab	6.8 ± 0.5 Ab
	Ruby Ribbons™	8.1 ± 0.8 Ca	27.1 ± 5.8 Ba	40.3 ± 3.5 Aa	50.5 ± 5.1 Aa
Pennisetum advena	-	10.6 ± 0.7 C	19.1 ± 1.4 B	17.6 ± 1.1 B	43.3 ± 3.0 A

Table 5.5. The percent of total leaves with red pigmentation in *Panicum virgatum* (switchgrass) and *Pennisetum advena* (purple fountaingrass) after 20 d exposure to four irradiance intensities. In each column, means with different letters are significantly different (Tukey's HSD, α=0.05).

	Taxa		
Irradiance ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	*Pennisetum advena*	*Panicum virgatum* Ruby Ribbons™	*Panicum virgatum* 'Heavy Metal'
75	18 ± 2 d	10 ± 3 c	0 ± 0 b
150	43 ± 2 c	31 ± 5 b	0 ± 0 b
300	75 ± 9 b	44 ± 4 b	0 ± 0 b
600	100 ± 0 a	83 ± 4 a	17 ± 4 a

Table 5.6. Total chlorophyll content (µg·cm^{-2}) of *Solenostemon scutellarioides* (coleus), *Panicum virgatum* (switchgrass), and *Pennisetum advena* (purple fountaingrass) after 20 d in response to four irradiance intensities (mean ± $_{SE}$). For each taxa, means within each row followed by different uppercase letters and means down each column followed by different lowercase letters are significantly different using Tukey's HSD ($\alpha = 0.05$).

Taxa	Cultivar	Irradiance (µmol·m^{-2}·s^{-1})			
		75	150	300	600
Solenostemon scutellarioides	'Dark Star'	30.2 ± 1.0 Aa	29.7 ± 1.1 Aa	25.6 ± 1.0 Ba	22.9 ± 1.5 Ba
	'Versa Burgundy to Green'	23.4 ± 1.7 Ab	23.3 ± 2.0 Ab	18.3 ± 1.6 Bb	15.4 ± 1.7 Bb
	'Twist and Twirl' green	20.2 ± 3.6 Ac	16.6 ± 2.8 Bcd	9.2 ± 2.0 Ccd	8.9 ± 2.1 Ccde
	'Dipt in Wine'	19.0 ± 2.4 Acd	17.2 ± 1.6 Ac	16.1 ± 1.9 Ab	18.0 ± 2.9 Ab
	'Solar Red'	14.9 ± 1.8 Aef	12.3 ± 0.8 ABef	10.5 ± 0.8 Bc	9.7 ± 0.8 Bcd
	'Twist and Twirl' red	14.8 ± 3.2 Bcde	21.2 ± 4.2 Ab	15.8 ± 3.0 Bb	18.6 ± 1.4 ABb
	'Versa Lime'	13.8 ± 0.9 Adef	10.8 ± 0.8 ABef	6.2 ± 0.8 BCcdef	5.3 ± 0.3 Cde
	'Versa Crimson Gold'	12.4 ± 1.1 Aefg	11.1 ± 1.1 ABde	6.8 ± 0.8 Bcde	8.2 ± 0.8 ABcd
	'Big Red Judy'	11.3 ± 0.5 Afgh	11.2 ± 0.8 Aef	10.5 ± 0.7 Ac	11.8 ± 0.7 Ac
	'LifeLime'	11.1 ± 0.4 Agh	8.3 ± 0.3 ABfg	6.0 ± 0.5 Bdef	3.5 ± 0.4 Cf
	'Royal Glissade'	7.9 ± 0.3 Ahi	6.4 ± 0.5 Ag	5.2 ± 0.6 Aef	6.6 ± 0.5 Aef
	'Sedona'	7.1 ± 0.4 Ai	5.0 ± 0.5 ABg	4.2 ± 0.5 ABf	2.6 ± 0.2 Bf
Panicum virgatum[z]	-	43.6 ± 1.7 A	34.3 ± 1.7 B	30.4 ± 2.1 B	22.2 ± 1.9 C
Pennisetum advena	-	41.6 ± 2.6 AB	47.8 ± 2.7 A	46.5 ± 2.5 A	35.7 ± 2.2 B

[z] Interaction of irradiance and cultivar not significant at $P < 0.05$ and means are pooled across cultivar.

Table 5.7. Anthocyanin content (μg·cm^{-2}) of five *Solenostemon scutellarioides* (coleus) cultivars after 25 d in response to four temperatures (mean ± $_{SE}$, n=12). Means within each column followed by different uppercase letters and means within each row followed by different lowercase letters are significantly different using Tukey's HSD ($\alpha = 0.05$).

Temperature (°C)	Cultivar				
	'Big Red Judy'	'LifeLime'	'Royal Glissade'	'Twist and Twirl' green	'Twist and Twirl' red
12	107.4 ± 4.5 Aa	1.1 ± 0.1 Ad	37.2 ± 4.0 Bc	2.5 ± 0.3 Ad	81.7 ± 5.1 Ab
18	72.9 ± 3.3 Ba	2.2 ± 0.1 Ac	55.3 ± 3.3 Ab	5.1 ± 0.7 Ac	71.1 ± 4.5 Aa
24	62.5 ± 2.7 Ba	2.0 ± 0.1 Ab	49.0 ± 4.2 ABa	5.2 ± 0.8 Ab	53.4 ± 3.9 Ba
30	65.9 ± 3.3 Ba	4.2 ± 0.3 Ac	57.6 ± 5.4 Aab	6.2 ± 0.6 Ac	48.7 ± 2.4 Bb

Table 5.8. Anthocyanin content (μg·cm^{-2}) of three *Solenostemon scutellarioides* (coleus) cultivars after 25 d in response to irradiance and temperature (mean ± $_{SE}$). The interaction between cultivar, temperature, and irradiance was non-significant at $P<0.05$ and values shown are for the significant two-way interactions. For each factor, means in each column followed by different uppercase letters and means in each row followed by different lowercase letters are significantly different at α = 0.05 (Tukey's HSD).

Factor	Level	Cultivar		
		'Big Red Judy'	'Royal Glissade'	'LifeLime'
Temperature[z]	12 °C	68.6 ± 4.2 Aa[z]	28.2 ± 1.3 Cb	1.9 ± 0.1 Ac
	18 °C	58.6 ± 1.8 Ba	42.1 ± 2.2 Bb	2.3 ± 0.1 Ac
	24 °C	52.4 ± 1.5 Ba	49.6 ± 1.8 Aa	2.1 ± 0.1 Ab
Irradiance	200 μmol·m^{-2}·s^{-1}	54.4 ± 1.4 Ba	36.1 ± 2.0 Bb	2.1 ± 0.1 Ac
	400 μmol·m^{-2}·s^{-1}	66.9 ± 3.2 Aa	43.1 ± 2.1 Ab	2.0 ± 0.1 Ac

[z] n = 24 (temperature x cultivar interaction) or n = 36 (irradiance x cultivar)

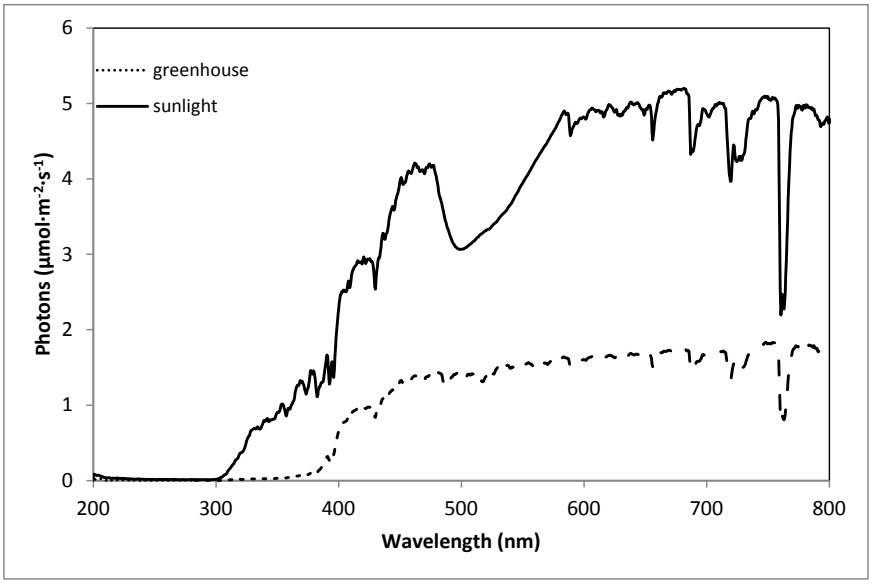

Fig. 5.1. Light transmission inside (dotted line) and outside (solid line) a greenhouse with a polycarbonate roof (St. Paul, MN). Light spectra were measured with a spectroradiometer on 6 Oct. 2011 at mid-day with no cloud cover. Total photosynthetically active radiation (*PAR*, 400-700 nm) was 435 and 1235 $\mu mol \cdot m^{-2} \cdot s^{-1}$ inside and outside, respectively (approximately 35% light transmission), and UV-B radiation (280-320 nm) was 0.2 and 3.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ inside and outside, respectively (6% transmission).

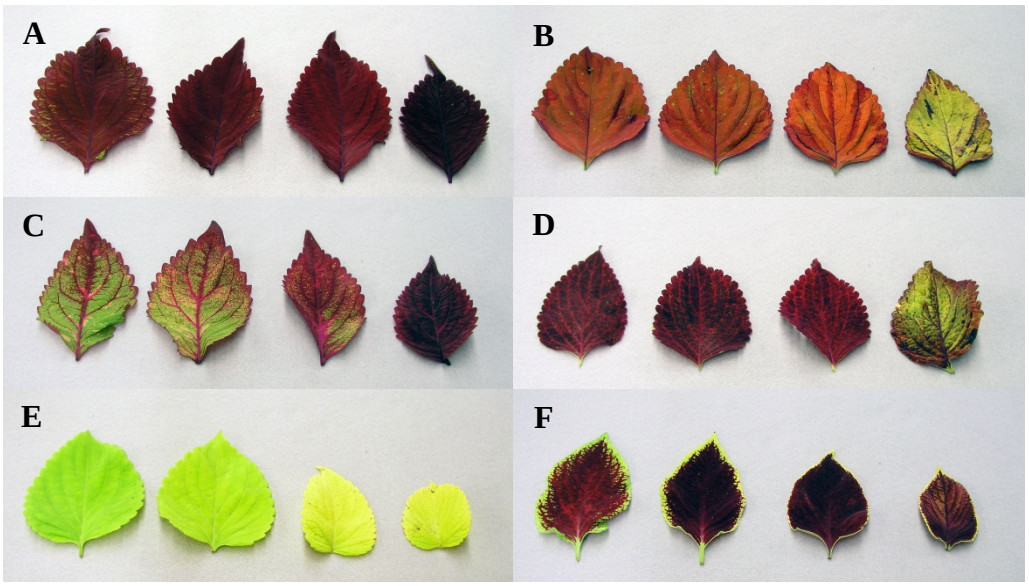

Fig. 5.2. Differential responses of selected *Solenostemon scutellarioides* (coleus) cultivars following 20 d exposure to four irradiance intensities (from left to right, 75, 150, 300, and 600 µmol·m^{-2}·s^{-1}). Anthocyanin content increased in 'Big Red Judy' (A) and 'Royal Glissade' (C) leaves as irradiance increased. In 'Sedona' (B), 'Solar Red' (D), and 'Versa Crimson Gold' (F), anthocyanin content increased from 75 to 300 µmol·m^{-2}·s^{-1}, but photobleaching occurred at 600 µmol·m^{-2}·s^{-1}. Anthocyanin content remained very low in green-leaved cultivars, e.g. 'LifeLime' (E).

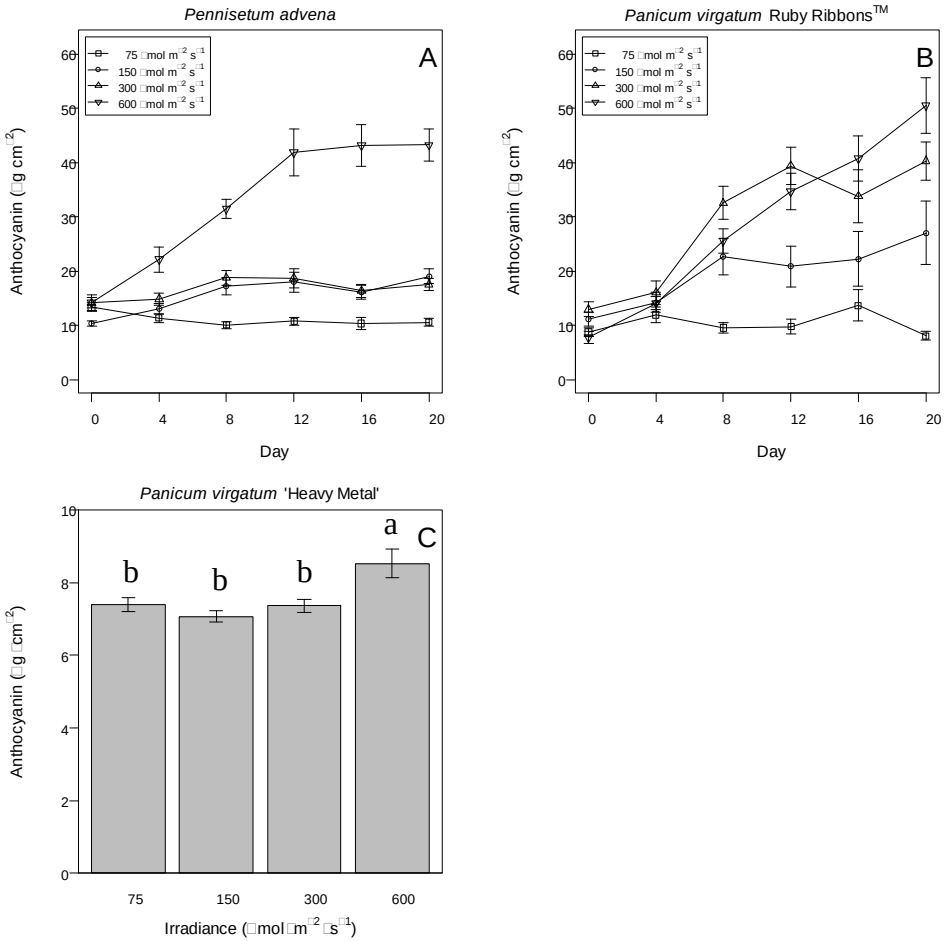

Fig. 5.3. Anthocyanin content (μg·cm^{-2}) of *Pennisetum advena* (purple fountaingrass, A) and *Panicum virgatum* (switchgrass, B and C) leaves in response to four irradiance intensities. Leaves were sampled at 4 d intervals, from 0 to 20 d. An interaction between irradiance and day occurred in *Pennisetum advena* (A) and *Panicum virgatum* Ruby Ribbons™ (B), while the main effect of irradiance in *Panicum virgatum* 'Heavy Metal' (C) is pooled across day.

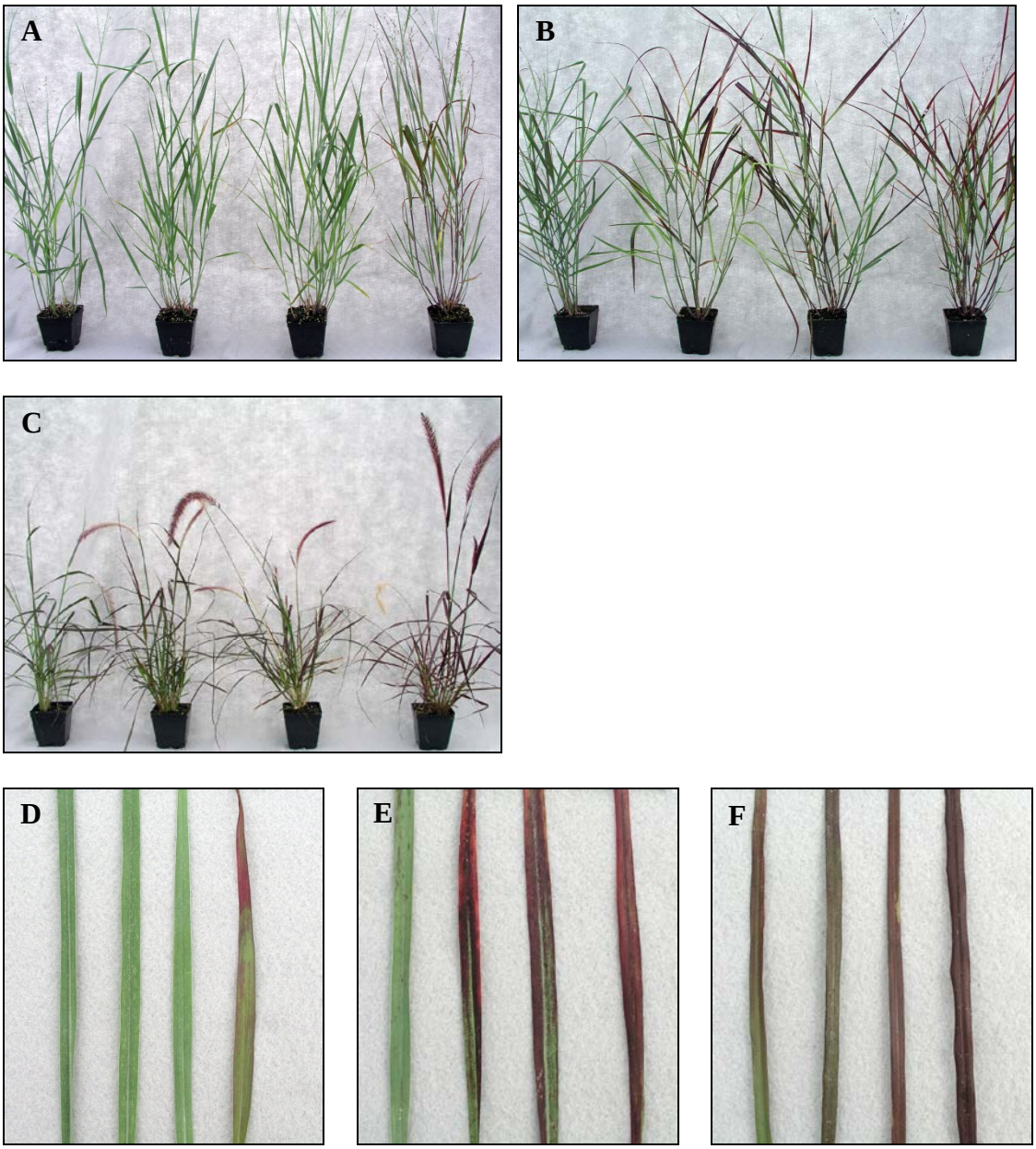

Fig. 5.4. Differential responses of *Panicum virgatum* 'Heavy Metal' (A and D) and Ruby Ribbons™ (B and E) and *Pennisetum advena* (C and F) leaves following 20 d exposure to four irradiance intensities (from left to right, 75, 150, 300, and 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$). An overall view of plants are shown in A-C, and close-ups of representative leaves shown in D-F.

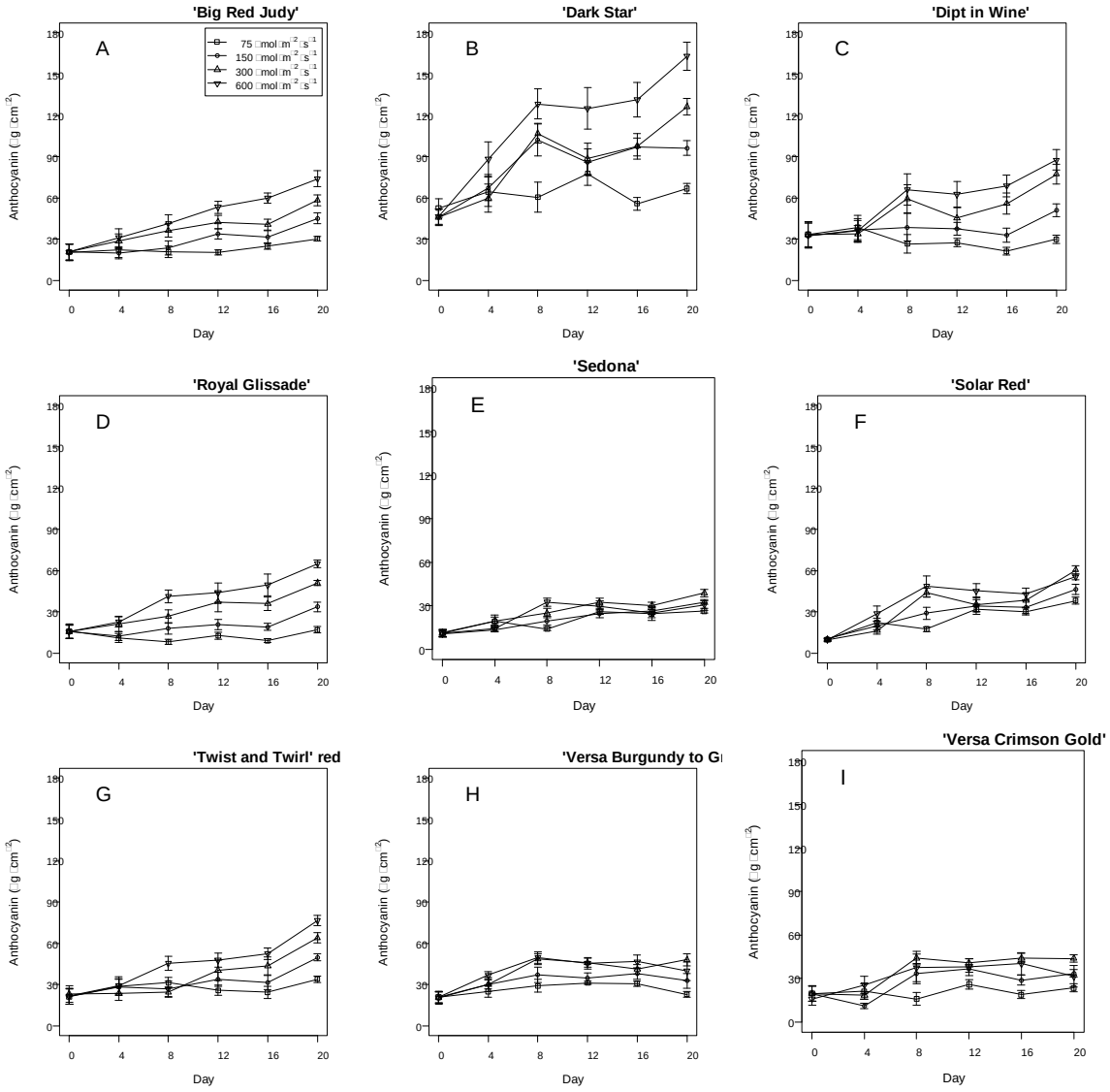

Fig. 5.5. Anthocyanin content (µg·cm^{-2}) of *Solenostemon scutellarioides* (coleus) cultivars in response to four irradiance intensities (µmol·m^{-2}·s^{-1}) at 4 d intervals, from 0 to 20 d: A) 'Big Red Judy', B) 'Dark Star', C) 'Dipt in Wine', D) 'Royal Glissade', E) 'Sedona', F) 'Solar Red', G) 'Twist and Twirl' red, H) 'Versa Burgundy to Green', and I) 'Versa Crimson Gold'. Irradiance levels: 75 (square), 150 (circle), 300 (triangle), and 600 µmol·m^{-2}·s^{-1} (inverted triangle).

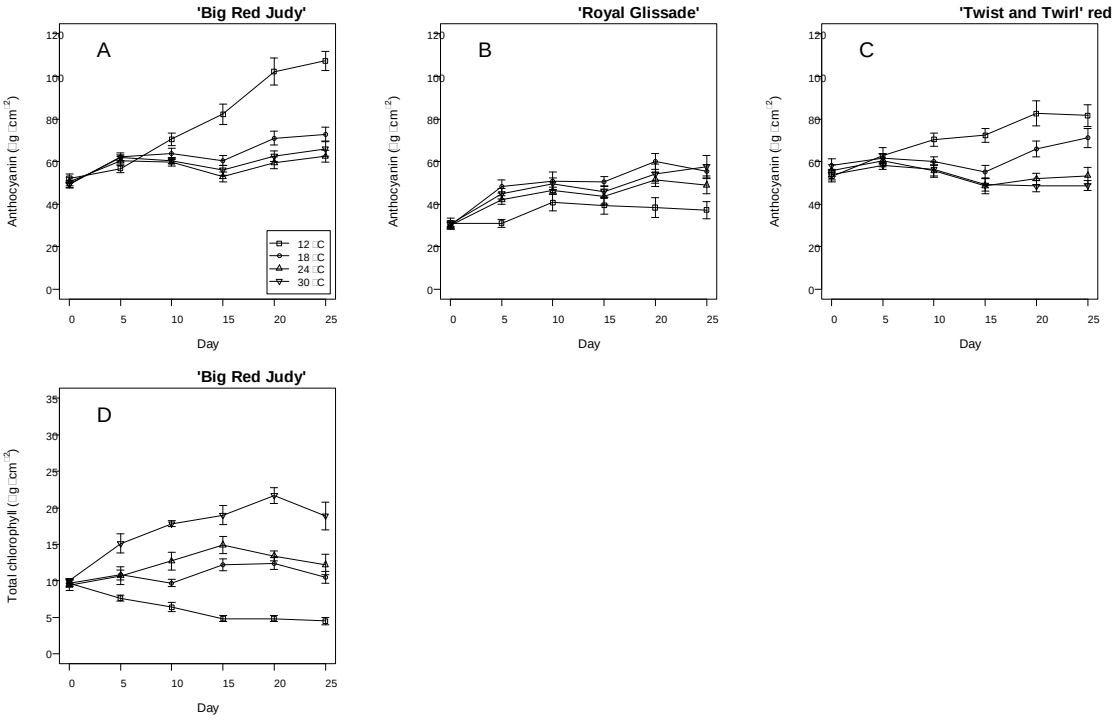

Fig. 5.6. Anthocyanin (A-C) and total chlorophyll (D) content ($\mu g \cdot cm^{-2}$) of *Solenostemon scutellarioides* (coleus) cultivars in response to four temperatures (°C) at 5 d intervals, from 0 to 25 d. Cultivars: 'Big Red Judy' (A, D), 'Royal Glissade' (B), and 'Twist and Twirl' red (C). Temperatures: 12 °C (square), 18 °C (circle), 24 °C (triangle), and 30 °C (inverted triangle).

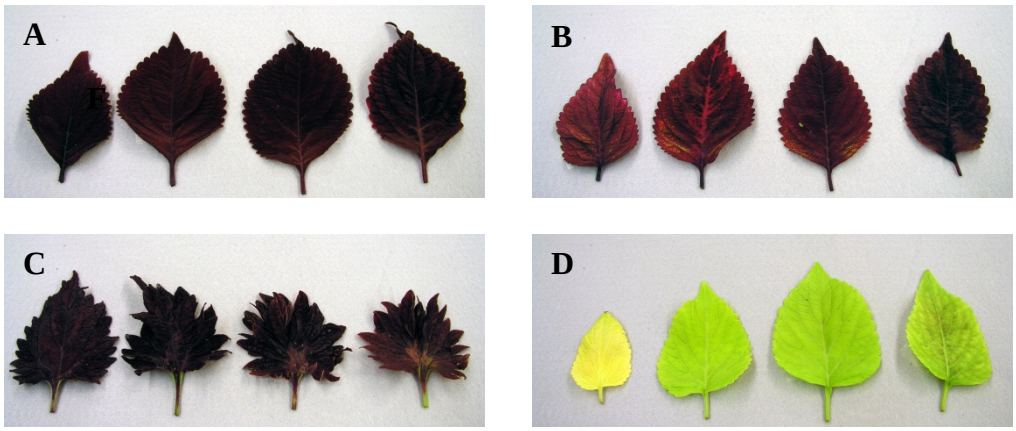

Fig. 5.7. Differential responses of *Solenostemon scutellarioides* (coleus) cultivars following 25 d exposure to four temperatures (from left to right, 12, 18, 24, and 30 °C): 'Big Red Judy' (A), 'Royal Glissade' (B),'Twist and Twirl' red (C), and 'LifeLime' (D).

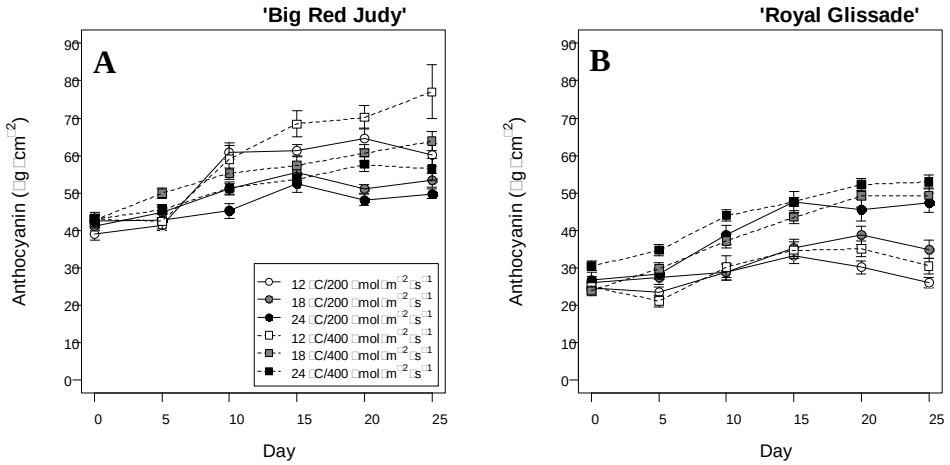

Fig. 5.8. Anthocyanin content (μg·cm^{-2}) of *Solenostemon scutellarioides* (coleus) cultivars in response to three temperatures (°C) and two irradiance intensities (μmol·m^{-2}·s^{-1}) at 5 d intervals, from 0 to 25 d: A) 'Big Red Judy' and B) 'Royal Glissade'. Temperatures: 12 (white), 18 (gray), or 24 °C (black). Irradiance: 200 (circle) or 400 (square) μmol·m^{-2}·s^{-1}.

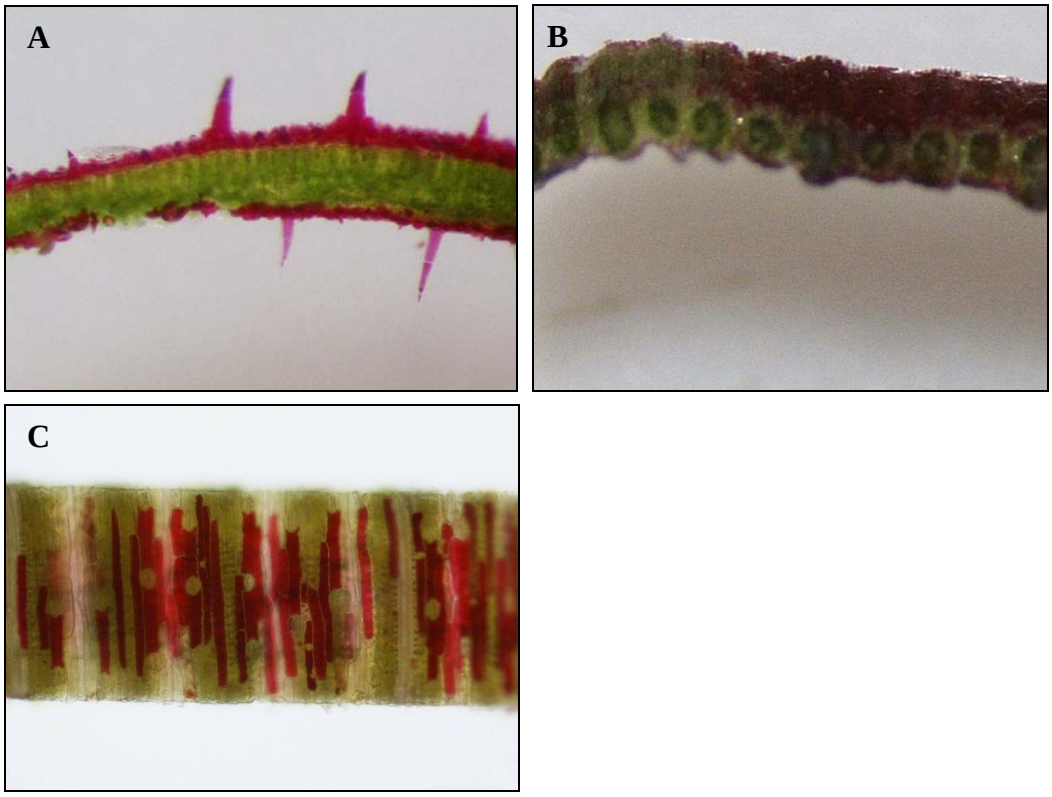

Fig. 5.9. *Solenostemon scutellarioides* (coleus, A) and *Panicum virgatum* Ruby Ribbons™ (switchgrass, B) leaf cross-sections, showing anthocyanin localization in the upper and lower epidermis. In *P. virgatum* Ruby Ribbons™, anthocyanin accumulation in the adaxial epidermis was a cell-specific response (C).

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Appendix A

Plant Growth Regulators Affect Leaf Pigmentation of *Solenostemon scutellarioides*

Solenostemon scutellarioides L. (Codd.) (coleus) 'Big Red Judy', 'Royal Glissade', and 'LifeLime' (red, red/green, and green-leaved cultivars, respectively) were sprayed with ancymidol (10 to 80 mg·L^{-1}), benzyladenine (75 to 600 mg·L^{-1}), chlormequat chloride (500 to 5000 mg·L^{-1}), daminozide (1,250 to 10,000 mg·L^{-1}), ethephon (250 to 2000 mg·L^{-1}), paclobutrazol (15 to 80 mg·L^{-1}), uniconazole (10 to 40 mg·L^{-1}) or deionized water. Anthocyanin content varied with cultivar but not treatment. In Expt. 1 (Spring 2011), foliar anthocyanin content in 'Big Red Judy' was greater than in 'Royal Glissade' (62.4 and 39.8 µg·cm^{-2}, respectively). Anthocyanin content was lower in Expt. 2 (Summer 2011), but 'Big Red Judy' (17.2 µg·cm^{-2}) and 'Royal Glissade' (17.0 µg·cm^{-2}) had higher anthocyanin content than 'LifeLime' (7.3 µg·cm^{-2}). Although total chlorophyll content in treated plants was similar to untreated plants (Expt. 2), it did vary between cultivars. Total chlorophyll content, in descending order, was 43.1 µg·cm^{-2} in 'LifeLime', 32.0 µg·cm^{-2} in 'Big Red Judy', and 19.4 µg·cm^{-2} in 'Royal Glissade'. The ratio of chlorophyll *a/b* was lower in 'Big Red Judy' than in the other two cultivars. The anthocyanin/chlorophyll ratio was greatest in 'Royal Glissade' (0.85), followed by 'Big Red Judy' (0.52), and 'LifeLime' (0.17). Overall, at the concentrations applied, the plant growth regulators had a minimal impact on anthocyanin and chlorophyll content in these three cultivars. However, differences in leaf pigmentation patterns were observed in

'Royal Glissade' plants treated with ancymidol, benzyladenine, daminozide, ethephon, and uniconazole.

{Chemical names used: ancymidol (α-cyclopropyl-α-(*p*-methoxyphenyl)-5-pyrimidinemethanol), benzyladenine (*N*-(phenylmethyl)-1*H*-purine-6-amine), chlormequat chloride [(2-chloroethyl) trimethylammonium chloride], daminozide [butanedioic acid mono (2,2-dimethylhydrazide)], ethephon [(2-chloroethyl) phosphonic acid], paclobutrazol [(±)-(R*,R*)-β-[(4-chlorophenyl)methyl]-α-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol], uniconazole [(E)-(+)-(S)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-pent-1-ene-3-ol)]}

Introduction

Plant growth regulators (PGRs) can impact plant growth and development, e.g. increase axillary branching, inhibit stem elongation, and reduce flower number (Davis and Andersen, 1989), and may affect anthocyanin concentration. Exogenous cytokinin applications increased anthocyanin content in *Arabidopsis thaliana* (Chen et al., 2006; Deikman and Hammer, 1995), but inhibited anthocyanin accumulation in *Zea mays* (Kim et al., 2006). Ethylene did not affect anthocyanin content in *A. thaliana* or grape leaf discs (Deikman and Hammer, 1995; Pirie and Mullins, 1976). Gibberellic acid (GA) inhibited anthocyanin accumulation in *Z. mays* leaves (Kim et al., 2006) and *Daucus carota* cell suspension cultures (Ilan and Dougall, 1992), but the application of GA

inhibitors (ancymidol, chlormequat choride, paclobutrazol, and uniconazole) effectively increased anthocyanin content in *D. carota* cell suspension cultures (A_{530}·ml^{-1}) (Ilan and Dougall, 1992). The objective of this study was to determine whether PGR applications (ancymidol, BA, chlormequat chloride, daminozide, ethephon, paclobutrazol, and uniconazole) affected anthocyanin content in coleus leaves. All PGRs used were GA inhibitors, except for BA (a cytokinin) and ethephon (an ethylene-producer).

Materials and Methods

Cuttings of coleus 'Big Red Judy', 'LifeLime', and 'Royal Glissade' (Pleasant View Gardens, Loudon, NH) were harvested from stock plants maintained in 16.5 cm diameter (1.3 L) pots filled with soilless media (LC-8; SunGro Horticulture, Bellvue, WA in a greenhouse (University of Minnesota, St. Paul, MN). Greenhouse set points were 22 °C day/18 °C night air temperatures [actual temperatures were 22.2 ± 0.8 °C/17.1 ± 0.3 °C in Expt. 1 (Spring 2011) and 22.2 ± 2.6 °C/18.5 ± 1.6 °C in Expt. 2 (Summer 2011)], ambient irradiance, plus 2 µmol·m^{-2}·s^{-1} lighting (incandescent bulbs) nightly from 2200-0200 $_{HR}$. Mean daily light integral (DLI, mol·m^{-2}·d^{-1}) was 13.9 ± 2.2 and 18.2 ± 5.5 (mean ± $_{SD}$) in Expt. 1 and 2, respectively]. Plants received night interruption lighting to keep them vegetative. Cuttings were trimmed to two nodes, placed in a 72-count cell tray (59 cm^3 volume) filled with LC-8 soilless media (SunGro Horticulture, Bellvue, WA) and placed under intermittent mist (5 s every 15 min) for 7 d until root formation occurred. Plants were then moved into the greenhouse and transplanted two weeks later into 10 cm

diameter (815 cm^3 volume) square pots filled with LC-8 soilless media. Plants were pruned to one node 10 d after transplant. Plants were watered as needed and fertilized twice weekly with 14.3 mM N from a 20N-4.4P-16.6K fertilizer (Peter's 20-10-20; The Scott's Co., Marysville, OH).

PGRs were applied 7 d after pinch as foliar sprays. In Expt. 1 (Spring 2011), three concentrations (low, medium, and high) of each PGR were applied, based on label recommendations and culture guidelines for efficacy in controlling plant growth. Treatments were the following: ancymidol (10, 20, and 40 $mg \cdot L^{-1}$), benzyladenine (BA; 75, 150, and 300 $mg \cdot L^{-1}$), chlormequat chloride (500, 1000, and 2000 $mg \cdot L^{-1}$), daminozide (1250, 2500, and 5000 $mg \cdot L^{-1}$), paclobutrazol (15, 30, and 60 $mg \cdot L^{-1}$), and uniconazole (10, 20, and 40 $mg \cdot L^{-1}$) (Fine Americas, Inc., Walnut Creek, CA); ethephon (250, 500, and 1000 $mg \cdot L^{-1}$; Southern Agricultural Insecticides, Inc., Palmetto, FL); and deionized water. Since only small differences in pigment concentrations were observed in Expt. 1, concentrations of all PGRs were increased for Expt. 2, except uniconazole, which was reduced due to excessive height reductions and small, curled leaves in all cultivars following the application of 40 $mg \cdot L^{-1}$. The following treatment concentrations were applied in Expt. 2 (Summer 2011): ancymidol (20, 40, and 80 $mg \cdot L^{-1}$), BA (150, 300, and 600 $mg \cdot L^{-1}$), chlormequat chloride (1250, 2500, and 5000 $mg \cdot L^{-1}$), daminozide (2,500; 5,000; and 10,000 $mg \cdot L^{-1}$), ethephon (500, 1000, and 2000 $mg \cdot L^{-1}$), paclobutrazol (20, 40, and 80 $mg \cdot L^{-1}$), uniconazole (5, 10, and 20 $mg \cdot L^{-1}$), and deionized water. Application rates were 2 quart/100 ft^2, except for ethephon (3 quart/100 ft^2). Three weeks after application, leaf discs were collected and anthocyanin and chlorophyll

content was quantified. Mean air temperatures (± $_{SD}$) for the interval between PGR application and data collection were 22.3 ± 1.9 °C day/16.9 ± 0.4 °C night in Expt. 1 and 28.3 ± 2.6 °C day/24.4 ± 2.7 °C night in Expt. 2. Mean DLIs were 21.0 ± 6.1 and 14.6 ± 2.4 mol·m^{-2}·d^{-1} (mean ± $_{SD}$) in Expt. 1 and 2, respectively.

Pigment quantification

Two leaf discs (each 0.3 cm^2), one for anthocyanin and one for chlorophyll content, were sampled from one leaf per plant. Leaf discs were collected from one leaf at the fourth node below the shoot apex. Each leaf disc was placed in a 1.7 mL microcentrifuge tube containing either 1 mL of 99:1 (v:v) methanol:HCl (for anthocyanin extraction) or 95% ethanol (for chlorophyll extraction) and placed in cold storage at 4 °C for 18 h. A 300 µL aliquot from each microcentrifuge tube was pipeted into a 96-well plate and absorbance was measured using a plate reader with monochromator optics (SpectraMax 190; Molecular Devices, Sunnyvale, CA). Absorbance for anthocyanin was measured at 530 nm, and chlorophyll was measured at 649 and 665 nm. Absorbance values were normalized to a 1 cm path length and a 1 cm^2 leaf area. Anthocyanin content was calculated as cyanidin-3-glucoside (Siegelman and Hendricks, 1958), and chlorophyll was calculated using equations published by Wintermans and De Mots (1965).

Experimental design/layout

The both experiments, the experimental design was a randomized complete block design (4 blocks, 3 cultivars, and 22 treatments), and the experimental unit was a leaf disc (n=4). Data were analyzed in SAS (PROC GLM; SAS 9.3, Cary, NC) and mean separation was conducted using Tukey's HSD (α=0.05) for sources of variation significant at P<0.05.

Results and Discussion

Anthocyanin content

Only cultivar affected anthocyanin content (Table A.1). In Expt. 1 (Spring 2011), 'Big Red Judy' (62.4 $\mu g \cdot cm^{-2}$) had higher anthocyanin content than 'Royal Glissade' (39.8 $\mu g \cdot cm^{-2}$) on a per unit area basis; anthocyanin content was not quantified in 'LifeLime'. In Expt. 2 (Summer 2011), anthocyanin content was similar in 'Big Red Judy' and 'Royal Glissade' (17.2 and 17.0 $\mu g \cdot cm^{-2}$, respectively) but higher than the anthocyanin content of 'LifeLime' (7.3 $\mu g \cdot cm^{-2}$; Table A.2). None of the PGRs, at the concentrations applied, influenced anthocyanin content compared to untreated plants. Because leaf area varied by treatment (data not shown), anthocyanin content in Expt. 2 was also calculated on a total leaf basis. Results were the same as those observed on a unit area basis. Total anthocyanins per leaf were similar in 'Big Red Judy' and 'Royal Glissade' (645 and 566 $\mu g \cdot leaf^{-1}$, respectively), and lower in 'LifeLime' (219 $\mu g \cdot leaf^{-1}$; Table A.2). The application of PGRs did not influence anthocyanin concentration per leaf in any treatment when compared to untreated plants.

In previous experiments, BA inhibited anthocyanin accumulation in *Zea mays* (Chen et al., 2006) but resulted in anthocyanin accumulation in *Arabidopsis thaliana* (Deikman and Hammer, 1995). In our experiments, BA did not appear to affect anthocyanin accumulation in coleus leaves. Ethylene did not affect anthocyanin content in *A.thaliana* or grape leaf discs (Deikman and Hammer, 1995; Pirie and Mullins, 1976), and our results concur with these studies. While the application of GA inhibitors (ancymidol, chlormequat choride, paclobutrazol, and uniconazole) to *D. carota* cell suspension cultures increased anthocyanin content (Ilan and Dougall, 1992), they did not influence anthocyanin content in our experiments at the concentrations applied.

The lack of differences between treatments observed here may be due to the concentrations of PGRs applied. In Expt. 1, we applied three concentrations (low, medium, and high) of each PGR, based on cultural guidelines and label recommendations for efficacy in controlling stem elongation in coleus or related ornamentals. These concentrations were increased in Expt. 2 (except for uniconazole), with minimal effect. Higher concentrations or higher application volumes may elicit a stronger response (either stimulatory or inhibitory to anthocyanin accumulation), but they would not normally be applied in commercial greenhouses due to excessive inhibition of stem elongation or phytotoxicity.

Anthocyanin was higher in the spring experiment (Expt. 1) than in the summer experiment (Expt. 2), even though higher concentrations of PGRs were applied in Expt. 2. Anthocyanin content in 'Big Red Judy' and 'Royal Glissade' was 3.6- and 2.3-fold higher, respectively, in Expt. 1. The decrease in Expt. 2 may have been due to higher

temperatures in Expt. 2; mean day and night air temperatures were 6.0 °C and 7.5 °C higher, respectively, in the summer compared to spring. Higher temperatures can increase the rate of anthocyanin degradation in pears, resulting in color loss (Steyn et al., 2005). Lower anthocyanin content occurred at higher temperature in grape berry skins as a result of lower mRNA levels of anthocyanin biosynthesis genes (Yamane et al., 2006). Lower anthocyanin content in field planting of lettuce was attributed to higher temperatures (Gazula et al., 2007). In a separate study utilizing these same coleus cultivars (Boldt et al., 2011), small differences in anthocyanin content were observed when they were grown at constant temperatures of 24 and 30 °C. Therefore, the increased temperature in summer partially, but not fully, explains the decreased anthocyanin content in Expt. 2.

Chlorophyll content

Chlorophyll content was quantified in Expt. 2 only. On a per area basis (μg·cm^{-2}), cultivar affected chl *a*, chl *b*, and total chlorophyll content (Table A.1). 'LifeLime' had the highest chlorophyll content (43.1 μg·cm^{-2}), followed by 'Big Red Judy' (32.0 μg·cm^{-2}) and 'Royal Glissade' (19.4 μg·cm^{-2}) had the lowest chlorophyll content (Table A.2). Although treatments affected chl *b* and total chlorophyll, none of the treatments were significantly different from untreated plants (Fig. A.1 and A.2).

Cultivar and PGR treatment interacted to affect chlorophyll content (Table A.3). In 'Royal Glissade' and 'Big Red Judy', no differences were observed between treated and untreated plants for chl *a*, chl *b*, or total chlorophyll content (data not shown).

Ancymidol and ethephon reduced leaf chlorophyll content of 'LifeLime'. Chl *a*, chl *b*, and total chlorophyll content were reduced following a 2000 $mg \cdot L^{-1}$ foliar spray of ethephon, and chl *a* and total chlorophyll content were reduced following an 80 $mg \cdot L^{-1}$ application of ancymidol. Chlorophyll content in 'LifeLime' was unaffected by other PGRs.

PGRs often increase chlorophyll content but may also have no effect or cause a decrease in chlorophyll content. The application of BA, a cytokinin, increased chlorophyll content in *Phaseolus vulgaris* (Adedipe et al., 1971; Naito et al., 1978) but did not impact chlorophyll content in our study. Ethephon reduced chl *a* and *b* in mandarin rind when applied at 250 $mg \cdot L^{-1}$ (El-Zeftawi, 1976), and in our study, chlorophyll content per leaf decreased in 'LifeLime' following a 2000 $mg \cdot L^{-1}$ ethephon foliar spray. However, chlorophyll content per leaf in 'Big Red Judy' and 'Royal Glissade' and chlorophyll content per unit area in all three cultivars was unaffected by ethephon at concentrations ranging from 250 to 2000 $mg \cdot L^{-1}$. It appears that chlorophyll content can decrease with high concentrations of ethephon; the extent of reduction, however, is cultivar dependent.

The application of PGRs often results in darker green leaves, but whether that results from increased chlorophyll biosynthesis or the same concentration of chlorophyll into a smaller leaf area is not known (Davis and Andersen, 1989). In our study, only ancymidol affected chlorophyll content compared to untreated plants. At 80 $mg \cdot L^{-1}$, ancymidol decreased chl *a* and total chlorophyll per leaf in 'LifeLime'. All other

treatments did not affect chlorophyll content in the three cultivars evaluated, which differs from results from most other studies.

While daminozide sprays on *Chrysanthemum morifolium* Ramat 'Yellow Reagan' and 'White Reagan' (Kazaz et al., 2010) and uniconazole drenches on dwarf Buford holly (Frymire and Cole, 1992) did not affect chlorophyll content as compared to untreated plants, others reported increased chlorophyll content following PGR application. Ancymidol (at 132 $mg \cdot L^{-1}$) increased chlorophyll content (per area) in *Helianthus annuus* by 75% (Starman et al., 1990). Daminozide, chlormequat chloride, paclobutrazol, and uniconazole sprays increased chlorophyll content in *Impatiens hawkeri* (New Guinea impatiens) 'Anguilla' and 'Papete' (Lee and Rho, 2000). Paclobutrazol drenches at 125 and 250 µg a.i. per 10-cm pot increased chlorophyll content in *Gylcine max* (Sankhla et al., 1985), and soil drenches of 30 to 90 µM paclobutrazol increased chlorophyll content per unit leaf area in *Zea mays*, although chlorophyll content per leaf decreased (Khalil and Rahman, 1995). Uniconazole (25 and 50 $mg \cdot L^{-1}$) foliar sprays increased chlorophyll content in *Brassica napus* (Zhou and Ye, 1996), and uniconazole sprays and drenches increased chlorophyll in pyracantha but not dwarf Buford holly (Frymire and Cole, 1992). Species and cultivars have different sensitivity thresholds to PGRs, and chlorophyll content, for the most part, was unaffected by PGR applications in coleus 'Big Red Judy', 'Royal Glissade', and 'LifeLime' at the concentrations tested. Chlorophyll content was influenced in 'LifeLime' only, with ancymidol or ethephon.

'Royal Glissade' and 'LifeLime' had the highest chl *a/b* ratios (3.6 and 3.4, respectively), while 'Big Red Judy' (3.1) had the lowest chl *a/b* ratio (Table A.2).

Although treatment was significant, none of the treatments had significantly different chlorophyll content from untreated plants (Table A.1, Fig. A.3). A lower chl *a/b* ratio is characteristic of shade-acclimated leaves (Lambers et al., 2008), and has been observed in anthocyanic leaves, most likely because anthocyanins act as a light filter. In juvenile *Quercus coccifera*, the chl *a/b* ratio was 2.85 in green leaves and 2.65 in red leaves (Manetas et al, 2003), and red-leaved coleus cultivars had lower chl *a/b* ratios than green-leaved cultivars (Henry et al., 2012). In our experiment, 'Big Red Judy', a cultivar with solid red leaves, had a lower chl *a/b* ratio than 'Royal Glissade', a cultivar with red and green speckled leaves, and 'LifeLime', a green-leaved cultivar. Anthocyanins in coleus leaves are present in upper (and lower) epidermal cells (data not shown), a location that would enable them to cause a light filtering effect on underlying chloroplasts.

Anthocyanin/chlorophyll ratio

The anthocyanin/chlorophyll ratio (Expt. 2) varied between cultivars (Table A.1). 'Royal Glissade' had the highest anth/chl ratio, followed by 'Big Red Judy', and 'LifeLime' (0.8, 0.5, and 0.2, respectively; Table A.2). Although 'Big Red Judy' and 'Royal Glissade' had similar anthocyanin contents, 'Royal Glissade' had higher anthocyanin/chlorophyll content as a result of its lower total chlorophyll content. 'LifeLime' had the lowest anthocyanin/chlorophyll ratio due to its low anthocyanin content.

Leaf pigmentation patterns

No aberrant leaf pigmentation patterns were observed in ‘Big Red Judy’ or ‘LifeLime’ after treatment with PGRs, but ‘Royal Glissade’ had divergent leaf patterns after treatment with ancymidol, BA, daminozide, ethephon, and uniconazole compared to untreated plants (Fig. A.4).

In untreated ‘Royal Glissade’ leaves, the basal region of mature leaves is green due to shading effects from newer, expanding leaves, and the unshaded, acropetal region is speckled red and green (Fig. A.4). Leaves are typically greener when grown under low light and redder when exposed to higher light intensities (Boldt et al., 2011). In this experiment, increased pink pigmentation in the major leaf veins occurred following application of ancymidol, daminozide, ethephon, or uniconazole at all concentrations (personal observation). In addition, the application of ancymidol, BA, daminozide, and ethephon resulted in non-uniform, colorless regions in many of the leaves, whereas the application of uniconazole resulted in uniform pigmentation across the leaf surface (Fig. A.4). New and novel patterns of coloration may be advantageous for colorful foliage plants such as coleus, however, it appears unlikely that PGRs will be a consistent source of this desirable variation.

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Table A.1. Analysis of variance for anthocyanin (anth) and chlorophyll (chl) content per unit area of three coleus cultivars ('Big Red Judy', 'LifeLime', and 'Royal Glissade') 3 weeks after the application of foliar plant growth regulator (PGR) sprays (Expt. 2). The experiment was a factorial (3 cultivars x 22 PGR treatments) arranged in a randomized complete block design (RCBD; n=4).

	Anth	Chl *a*	Chl *b*	Total Chl	Chl *a*/*b*	Anth/Chl
Block	***[z]	***	*	***	***	***
Cultivar	***	***	***	***	***	***
Treatment	NS	NS	***	*	***	NS
C x T	NS	NS	NS	NS	NS	NS

[z] NS, *, and *** Nonsignificant or significant at $P \leq 0.05$ or 0.001, respectively.

Table A.2. Anthocyanin and chlorophyll content in three coleus cultivars 3 weeks after the application of foliar plant growth regulator (PGR) sprays (Expt. 2). The interaction between cultivar and treatment were not significant at $P<0.05$, and the main effects of cultivar are pooled across all treatments (values are mean $\pm_{SE,}$ n=88). Means in each row followed by different letters are significantly different (Tukey's HSD, $\alpha=0.05$).

Trait	Cultivar		
	'Big Red Judy'	'Royal Glissade'	'LifeLime'
Anthocyanin ($\mu g \cdot cm^{-2}$)	17.2 ± 1.2 a	17.0 ± 1.5 a	7.3 ± 0.1 b
Chlorophyll *a* ($\mu g \cdot cm^{-2}$)	23.9 ± 0.6 b	15.0 ± 0.4 c	33.2 ± 0.7 a
Chlorophyll *b* ($\mu g \cdot cm^{-2}$)	8.1 ± 0.2 b	4.4 ± 0.2 c	9.9 ± 0.3 a
Total chlorophyll ($\mu g \cdot cm^{-2}$)	32.0 ± 0.7 b	19.4 ± 0.5 c	43.1 ± 1.0 a
Chlorophyll *a/b*	3.1 ± 0.1 b	3.6 ± 0.1 a	3.4 ± 0.1 a
Anthocyanin/Chlorophyll	0.5 ± 0.0 b	0.8 ± 0.1 a	0.2 ± 0.0 c

Table A.3. Analysis of variance for total anthocyanin (anth) and chlorophyll (chl) content per leaf of three coleus cultivars ('Big Red Judy', 'LifeLime', and 'Royal Glissade') 3 weeks after the application of foliar plant growth regulator (PGR) sprays (Expt. 2). The experiment was a factorial (3 cultivars x 22 PGR treatments) arranged in a randomized complete block design (RCBD; n=4).

	Anth	Chl a	Chl b	Total chl
Block	***[z]	***	Ns	***
Cultivar	***	***	***	***
Treatment	**	***	***	***
C x T	[NS]	**	**	**

[z] NS, **, and *** Nonsignificant or significant at $P \leq 0.01$ or 0.001, respectively.

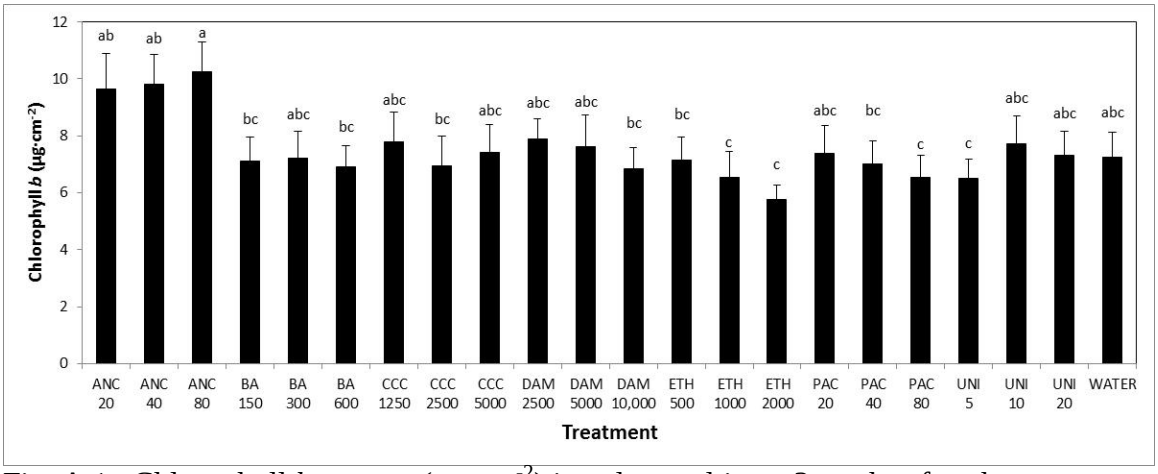

Fig. A.1. Chlorophyll *b* content (µg·cm^{-2}) in coleus cultivars 3 weeks after the application of plant growth regulators (PGRs). Values (mean ± $_{SE}$) for each PGR treatment are pooled across cultivar (n=12). Each PGR was applied at three concentrations (low, medium, and high) based on cultural guidelines and label recommendations. Abbreviations: ANC, ancymidol; BA, benzyladenine; CCC, chlormequat chloride; DAM, daminozide; ETH, ethephon; PAC, paclobutrazol; and UNI, uniconazole.

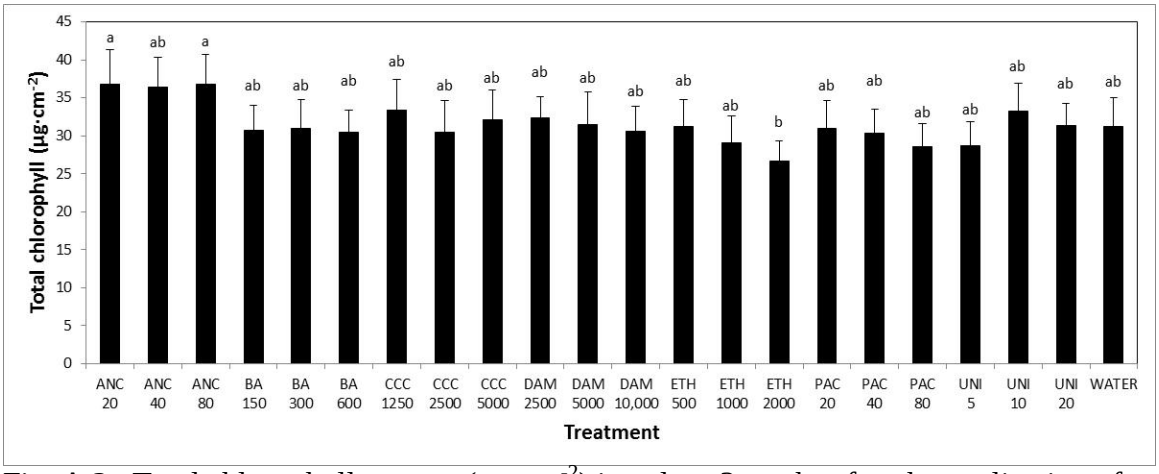

Fig. A.2. Total chlorophyll content (µg·cm^{-2}) in coleus 3 weeks after the application of plant growth regulators (PGRs). Values (mean ± $_{SE}$) for each PGR treatment are pooled across cultivar (n=12). Each PGR was applied at three concentrations (low, medium, and high) based on cultural guidelines and label recommendations. Abbreviations: ANC, ancymidol; BA, benzyladenine; CCC, chlormequat chloride; DAM, daminozide; ETH, ethephon; PAC, paclobutrazol; and UNI, uniconazole.

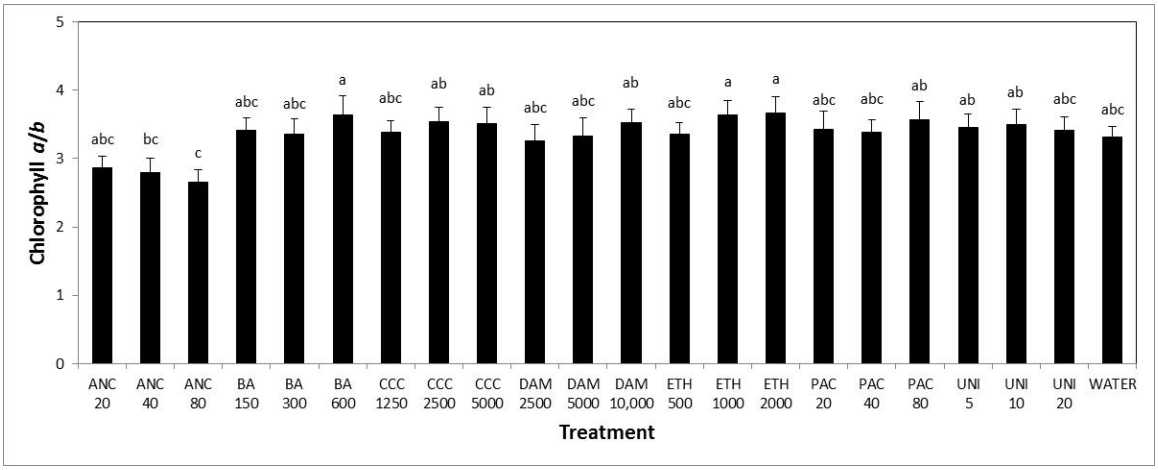

Fig. A.3. Chlorophyll *a/b* ratio in coleus 3 weeks after the application of plant growth regulators (PGRs). Values (mean ± $_{SE}$) for each PGR treatment are pooled across cultivar (n=12). Each PGR was applied at three concentrations (low, medium, and high) based on cultural guidelines and label recommendations. Abbreviations: ANC, ancymidol; BA, benzyladenine; CCC, chlormequat chloride; DAM, daminozide; ETH, ethephon; PAC, paclobutrazol; and UNI, uniconazole.

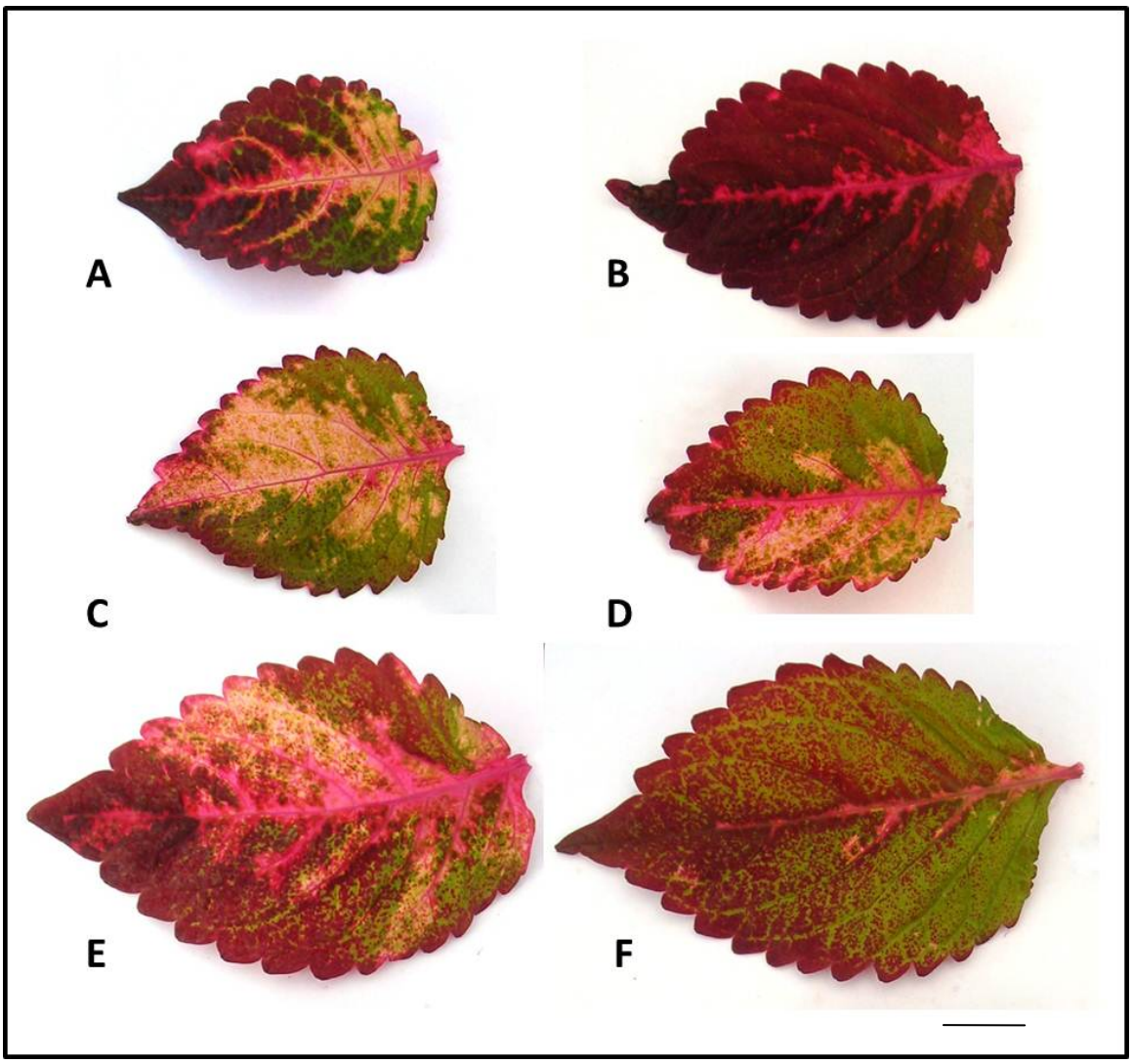

Fig. A.4. Plant growth regulators (PGRs) can affect leaf pigmentation patterns in coleus 'Royal Glissade'. Pictures were taken 3 weeks after the application of PGRs. In untreated plants (F), the basal region of mature leaves is green due to shading effects from newer, expanding leaves, and the acropetal region is speckled red and green. Divergent pigmentation patterns were observed following treatment with all PGRs, except chlormequat chloride and paclobutrazol. While variations in the pigmentation pattern occurred at all three application concentrations of each PGR, representative leaves from the medium application concentration are shown: A) ancymidol, 40 $mg \cdot L^{-1}$, B) uniconazole, 10 $mg \cdot L^{-1}$, C) benzyladenine, 300 $mg \cdot L^{-1}$, D) ethephon, 1000 $mg \cdot L^{-1}$, E) daminozide, 5000 $mg \cdot L^{-1}$, and F) untreated plants. Scale = 1 cm.

Appendix B

Overview of Chlorophyll Fluorescence: A Primer

A photon of light intercepted by a leaf can be 1) absorbed by leaf pigments, 2) transmitted through the leaf, 3) or reflected off the leaf. A photon (quantum) of light absorbed by chlorophyll transfers its energy to a valence electron, raising it to a higher (excited) energy state. When the electron returns to the ground state, it passes on the absorbed energy to one of three paths: 1) photochemistry, 2) heat dissipation, or 3) fluorescence. It can be assumed that the total of the three potential paths is equal to 1.

$$\text{Photochemistry (P)} + \text{Heat dissipation (H)} + \text{Fluorescence (F)} = 1 \qquad \text{(Eq. 1)}$$

In the dark, when a brief flash of light occurs, almost all of the absorbed energy will be directed towards photochemistry (P = 1). As the light intensity of the pulse increases, the proportion of energy directed towards P will decrease and H and F will increase. At saturating light levels, any further increase in irradiance will not result in a further increase in photochemistry, i.e. P = 0, because all of the electron acceptors in the electron transport chain will be in a reduced state. Consequently, H and F will be at their maximum values (H_m and F_m) and $H_m + F_m = 1$.

Provided that the ratio between heat and fluorescence does not change when a short, saturating pulse of light is provided, then the ratio H/F is equal to H_m/F_m.

Substituting for H_m:

$$H_m = 1 - F_m$$

$$H/F = H_m/F_m = (1-F_m)/F_m$$

Rearrangment of $H/F= (1-F_m)/F_m$ yields $H = F(1-F_m)/F_m$

Substitution and re-arrangement of Eq. 1 yields: $P = (F_m-F)/F_m$

In a dark-adapted leaf, fluorescence measurements collected in the dark will be:

$$P_{dark} = (F_m-F_o)/F_m = F_v/F_m \quad \text{(Eq. 2)}$$

The minimal fluorescence in a dark-adapted leaf is denoted as F_o and the variable fluorescence (F_v) is the difference between the maximal and minimal fluorescence (Fig. B.1). This is the proportion of absorbed photons directed towards photochemistry in a dark-adapted leaf. In healthy leaves, F_v/F_m is typically about 0.83 (Maxwell and Johnson, 2000). In dark-adapted leaves, electron acceptors will be "empty" (oxidized) and non-photochemical quenching mechanisms (such as the xanthophyll cycle) will be minimal. Thus, P_{dark} is known as the maximum quantum yield

At non-saturating irradiances, some or all of the election acceptors will be partially or completely reduced, and non-photochemical quenching mechanisms be activated. Eq. 2 becomes

$$P_{light} = (F_m'-F_s)/F_m' = \Delta F/F_m' = \Phi_{PSII} \quad \text{(Eq. 3)}$$

F_m' is the maximal light-adapted fluorescence, F_s is the steady-state fluorescence, and ΔF is the difference between F_m' and F_s. This is known as Φ_{PSII}, the effective quantum yield. It is the proportion of absorbed quanta directed towards photochemistry in a stable, light-adapted leaf. F_m' will be lower in magnitude than F_m because some of the absorbed quantum energy in a light-adapted leaf will be directed towards non-photochemical quenching rather than fluorescence under a brief, saturating pulse of irradiance.

Chlorophyll fluorescence measurements can also be used to estimate the flow of electrons through the photosystems:

$$ETR = [(F_m'-Fs)/F_m']f\ I\ \alpha_{leaf} \quad \text{(Eq. 4)}$$

with f = the fraction of absorbed quanta directed to PSII (assumed to be 0.5), I = incident irradiance ($\mu mol\ m^{-2}\ s^{-1}$), and α_{leaf} = the proportion of incident irradiance absorbed by the leaf (assumed to be 0.8 unless the leaf has protective mechanisms, such as a thick cuticle, in which case it would be lower).

Photochemical quenching (qP) includes both photosynthesis and photorespiration. It will be greatest under low irradiances and will decrease as irradiance increases. It can be calculated from chlorophyll fluorescence measurements:

$$qP = (F_m'-F_s)/(F_m'-F_o') \quad \text{(Eq. 5)}$$

F_o' is measured following a dark pulse in a light-adapted leaf in order to "drain" PSII of elections. This value gives an indication of the degree of oxidation of Q_A, the initial electron acceptor for PSII (Smillie and Hetherington, 1999). When qP = 1 (no difference between F_s and F_o'), Q_A is fully oxidized ("open" to accept electrons). A value of qP < 1 indicates that some Q_A molecules are in their reduced state ("closed" and unable to accept electrons).

Non-photochemical quenching includes protection mechanisms (such as heat dissipation via the xanthophyll cycle), and increases in value as irradiance increases., in part, due to the reduced ability of photosystems to accept energy from absorbed quanta. It can be reported as

$$qN = (F_m\text{-}F_m')/(F_m\text{-}F_o'), \quad \text{or} \qquad \text{(Eq. 6)}$$

$$NPQ = (F_m\text{-}F_m')/F_m' \qquad \text{(Eq. 7)}$$

For all of these calculated fluorescence parameters, values are relative measurements. It is important when collecting both dark and light-adapted fluorescence measurements to use the same exact leaf surface area so that values can be accurately calculated. With the LI-6400 portable gas exchange system, it is possible to collect simultaneous gas exchange and fluorescence measurements from leaves. This is useful because it allows one to compare net photosynthetic rates based on CO_2 intake and electron transport in the leaf. In C_3 and C_4 plants, the trends in both photosynthesis and electron transport should be similar in response to irradiance. In CAM plants, however,

they will differ due to the temporal separation of CO_2 uptake (uptake and storage in cell vacuoles as organic acids during the night) and carbon fixation (production of reduced cofactors, ATP and fixed carbon, i.e. sugars, during the day).

The light source in the leaf chamber fluorometer (LCF), the cuvette head attachment used with the LI-6400*XT* for fluorescence, is a combination of blue, red, and far-red light-emitting diodes (LEDs). The blue and red LEDs are used in combination for driving photosynthesis (the ratio is user defined); blue LEDs have a peak centered at 470 nm and red LEDs have a peak centered at 630 nm. The background light level is referred to as "actinic light". Red LEDs and are used to provide saturating light pulses for fluorescence measurements, and a detector measures fluorescence at wavelengths >700 nm. Far-red light is used when measuring F_o'; the actinic light is briefly turned off and far-red light (740 nm) is used to drive PSI and drain electrons from PSII.

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Smillie, R.M. and S.E. Hetherington. 1999. Photoabatement by anthocyanin shields photosynthetic systems from light stress. Photosynthetica 36:451-463.

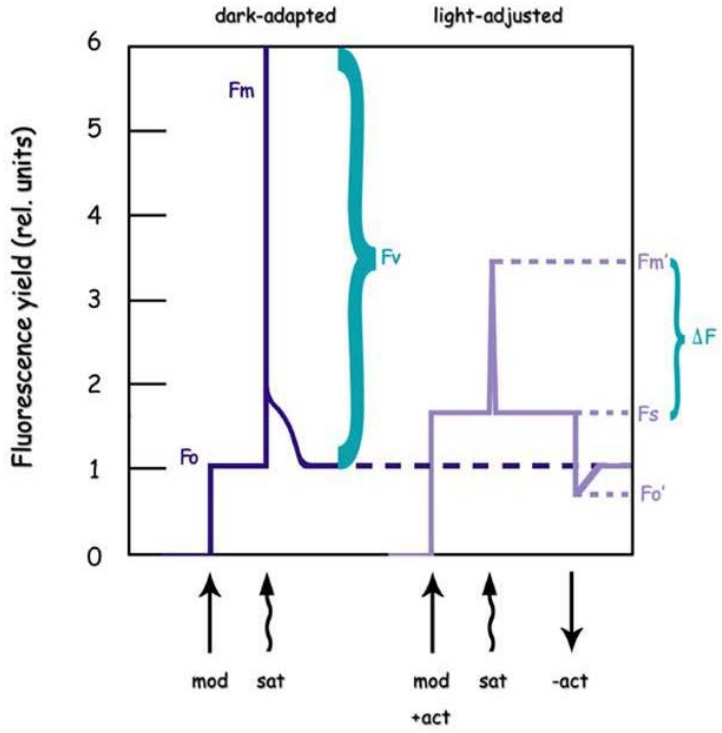

Fig. B.1. Overview of chlorophyll fluorescence measurements. On dark-adapted leaves, minimum (F_o) and maximum (F_m) fluorescence values are recorded, and Fv can be calculated as the difference between F_m and F_o. On light-adapted leaves, minimum steady state (F_s) and maximum (F_m') fluorescence values are recorded during a saturating light pulse, and the minimum fluorescence (F_o') is measured following a brief dark period (from LICOR, 2008).

Appendix C

Supplementary Tables and Figures

Table C.1. Analysis of variance of nine coleus (*Solenostemon scutellarioides*) and two switchgrass (*Panicum virgatum*) cultivars in response to irradiance (Chapter 3). Each species was analyzed separately. The experimental design was a two-way factorial (cultivar, PAR) arranged in a randomized complete block design with repeated measures.

Species	Source	df	A_{max} (leaf area)	A_{max} (ambient)	A_{max} (FW)	A_{max} (DW)	A_{max} (CHL)	Φ_{PSII}	F_v'/F_m'	ETR	qP	qN	NPQ
Coleus	Blk	4	0.15	0.23	0.19	0.18	0.20	0.06	0.06	0.06	0.05	0.25	0.20
	Cv	8	<0.0001	<0.0001	0.0003	0.0004	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	PAR	10	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Cv x PAR	80	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Switchgrass	Blk	4	0.80	0.07	0.78	0.77	0.84	0.83	0.67	0.79	0.94	0.28	0.83
	Cv	1	0.05	0.37	0.03	0.03	0.27	0.21	0.22	0.18	0.21	<0.0001	0.01
	PAR	10	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Cv x PAR	10	<0.0001	0.22	<0.0001	<0.0001	0.49	0.19	0.0004	0.0002	<0.0001	0.008	0.0002

Table C.2. Analysis of variance of nine coleus (*Solenostemon scutellarioides*) and two switchgrass (*Panicum virgatum*) cultivars in response to CO_2 (Chapter 3). Each species was analyzed separately. The experimental design was a two-way factorial (cultivar, PAR) arranged in a randomized complete block design with repeated measures.

Species	Source	df	A_{max} (leaf area)	A_{max} (ambient)	A_{max} (FW)	A_{max} (DW)	A_{max} (CHL)	Φ_{PSII}	F_v'/F_m'	ETR	qP	qN	NPQ
Coleus	Blk	4	0.004	0.02	0.005	0.007	0.03	0.002	<0.0001	0.002	0.08	0.007	0.01
	Cv	8	<0.0001	0.52	0.0006	0.0005	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	CO_2	12	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Cv x CO_2	96	<0.0001	0.04	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Switchgrass	Blk	4	0.80	0.91	0.80	0.81	0.56	0.79	0.60	0.79	0.82	0.45	0.04
	Cv	1	0.08	0.70	0.04	0.04	0.08	0.46	0.62	0.45	0.41	0.04	0.003
	CO_2	12	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Cv x CO_2	12	<0.0001	0.91	<0.0001	<0.0001	0.004	0.003	<0.0001	0.003	0.94	<0.0001	0.03

Table C.3. Analysis of variance of nine coleus (*Solenostemon scutellarioides*) and two switchgrass (*Panicum virgatum*) cultivars in response to temperature (Chapter 3). Each species was analyzed separately. The experimental design was a two-way factorial (cultivar, *PAR*) arranged in a randomized complete block design with repeated measures.

Species	Source	df	A_{max} (leaf area)	A_{max} (ambient)	A_{max} (FW)	A_{max} (DW)	A_{max} (CHL)	F_v/F_m	Φ_{PSII}	ETR	qP	qN	NPQ
Coleus	Blk	4	0.19	0.03	0.14	0.15	0.07	0.34	0.20	0.19	0.13	0.40	0.52
	Cv	8	<0.0001	0.10	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	<0.0001
	Temp	4	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Cv x Temp	32	<0.0001	0.006	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.007	0.0005	0.0004
Switchgrass	Blk	4	0.37	0.22	0.37	0.38	0.41	0.91	0.21	0.21	0.18	0.44	0.47
	Cv	1	0.01	0.24	0.006	0.004	0.06	0.23	0.004	0.0004	0.07	0.004	0.004
	Temp	4	<0.0001	0.0003	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Cv x Temp	4	0.005	0.13	0.002	0.001	0.55	0.63	0.003	0.003	0.01	0.09	0.002

Table C.4. Analysis of variance of 12 *Solenostemon scutellarioides* (coleus) cultivars, two *Panicum virgatum* (switchgrass) cultivars, and *Pennisetum advena* (purple fountaingrass) in response to irradiance (Chapter 5). The experimental design was a three-way factorial (irradiance, cultivar, and day) arranged in a split plot design, with irradiance ($\mu mol \cdot m^{-2} \cdot s^{-1}$) as the main plot, cultivar as the subplot, and repeated measures (day) on the subplot. Each species was analyzed separately.

Species	Source	df	Anthocyanin	% Anth	Total Chl	% Tot Chl	Chl *a*/*b*	Anth/Chl ratio
Solenostemon scutellarioides	Rep	2	<0.0001	<0.0001	<0.0001	0.04	0.25	0.002
	Irradiance	3	<0.0001	0.0007	0.0004	0.22	0.27	0.03
	Cultivar	11	<0.0001	<0.0001	<0.0001	0.01	<0.0001	<0.0001
	I x C	33	0.35	0.23	0.99	0.99	0.63	0.16
	Day	5	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	I x D	15	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	C x D	55	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	I x C x D	165	<0.0001	<0.0001	0.09	0.06	<0.0001	<0.0001
Panicum virgatum	Rep	2	0.24	0.17	<0.0001	0.001	0.009	0.40
	Irradiance	3	0.0003	<0.0001	<0.0001	0.17	<0.0001	0.04
	Cultivar	1	<0.0001	<0.0001	<0.0001	0.04	<0.0001	0.0006
	I x C	3	0.0006	<0.0001	0.28	0.72	0.0001	0.10
	Day	5	<0.0001	<0.0001	0.04	0.02	0.0004	0.0001
	I x D	15	<0.0001	<0.0001	<0.0001	0.40	<0.0001	0.02
	C x D	5	<0.0001	<0.0001	0.10	0.01	<0.0001	<0.0001
	I x C x D	15	<0.0001	<0.0001	0.77	0.99	0.01	0.03
Pennisetum advena	Rep	2	0.08	0.11	0.40	0.002	0.11	0.18
	Irradiance	3	<0.0001	<0.0001	0.03	0.11	0.24	0.0005
	Day	5	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	I x D	15	<0.0001	<0.0001	0.23	0.62	0.08	<0.0001

Table C.5. Analysis of variance of 12 *Solenostemon scutellarioides* (coleus) cultivars, two *Panicum virgatum* (switchgrass) cultivars, and *Pennisetum advena* (purple fountaingrass) in response to irradiance after 20 d (Chapter 5). The experimental design was a two-way factorial (irradiance, cultivar) arranged in a split plot design, with irradiance ($\mu mol \cdot m^{-2} \cdot s^{-1}$) as the main plot and cultivar as the subplot. Each species was analyzed separately.

Species	Source	df	Anthocyanin	% Anth	Total Chl	% Tot Chl	Chl *a*/*b*	Anth/Chl ratio	% Red leaves
Solenostemon	Rep	2	0.0005	0.002	0.0003	0.14	0.04	0.01	-
scutellarioides	Irradiance	3	<0.0001	0.02	0.002	0.81	0.047	0.01	-
	Cultivar	11	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-
	I x C	33	<0.0001	<0.0001	0.0007	<0.0001	<0.0001	<0.0001	-
Panicum	Rep	2	0.08	0.26	0.37	<0.0001	0.09	0.43	0.68
virgatum	Irradiance	3	<0.0001	<0.0001	0.0005	0.001	0.38	0.23	0.02
	Cultivar	1	<0.0001	<0.0001	<0.0001	0.10	<0.0001	0.0002	<0.0001
	I x C	3	<0.0001	<0.0001	0.09	0.12	<0.0001	0.10	<0.0001
Pennisetum	Rep	2	0.63	0.91	0.01	<0.0001	<0.0001	0.95	0.003
advena	Irradiance	3	<0.0001	<0.0001	0.003	0.08	<0.0001	<0.0001	<0.0001

Table C.6. Percent change in anthocyanin content (after 20 d) of *Solenostemon scutellarioides* (coleus), *Panicum virgatum* (switchgrass), and *Pennisetum advena* (purple fountaingrass) in response to irradiance (mean ± $_{SE}$, n=15). For each taxa, means within each row followed by different uppercase letters or means down each column followed by different lowercase letters are significantly different using Tukey's HSD ($\alpha = 0.05$).

Taxa	Cultivar	Irradiance ($\mu mol \cdot m^{-2} \cdot s^{-1}$)			
		75	150	300	600
Solenostemon scutellarioides	'Royal Glissade'	199 ± 58 Dbcd	541 ± 121 Ca	1050 ± 202 Ba	2176 ± 168 Aa
	'Twist and Twirl' red	104 ± 49 Bbcdef	171 ± 56 Bbcdef	569 ± 175 Abc	813 ± 185 Ab
	'Big Red Judy'	387 ± 97 Ca	401 ± 82 BCab	629 ± 130 ABb	780 ± 161 Ab
	'Solar Red'	272 ± 16 Bab	345 ± 27 ABbcd	535 ± 32 Abc	491 ± 53 ABc
	'Dipt in Wine'	58 ± 23 Bdef	216 ± 61 ABcdef	325 ± 60 Ade	413 ± 78 Acd
	'Dark Star'	70 ± 31 Bcdef	169 ± 47 ABef	225 ± 44 ABef	342 ± 74 Ad
	'Sedona'	242 ± 46 Aabc	374 ± 62 Abc	443 ± 78 Acd	304 ± 73 Ade
	'Versa Crimson Gold'	117 ± 51 Bbcde	194 ± 65 ABbcde	349 ± 116 Abcd	130 ± 49 ABde
	'Versa Burgundy to Green'	41 ± 29 Abcdef	93 ± 41 Adef	173 ± 29 Adef	112 ± 33 Ade
	'Twist and Twirl' green	-9 ± 16 Aef	-9 ± 12 Ag	-1 ± 20 Ag	72 ± 29 Af
	'LifeLime'	-10 ± 4 Af	-8 ± 10 Ag	-27 ± 5 Ag	-3 ± 10 Ag
	'Versa Lime'	-16 ± 11 Adef	-23 ± 9 Afg	-21 ± 12 Afg	-18 ± 8 Aef
Panicum virgatum	'Heavy Metal'	1 ± 10 Aa	-3 ± 9 Aa	-30 ± 5 Ab	-13 ± 9 Ab
	Ruby Ribbons™	-1 ± 9 Ca	189 ± 64 BCa	244 ± 33 Ba	682 ± 110 Aa
Pennisetum advena	-	-15 ± 8 B	87 ± 15 B	33 ± 12 B	259 ± 50 A

Table C.7. Anthocyanin/chlorophyll ratio after 20 d of *Solenostemon scutellarioides* (coleus) and *Pennisetum advena* (purple fountaingrass) in response to irradiance (mean ± $_{SE}$, n=15)[z]. For each taxa, means within each row followed by different uppercase letters or means down each column followed by different lowercase letters are significantly different using Tukey's HSD ($\alpha = 0.05$).

Taxa	Cultivar	Irradiance ($\mu mol \cdot m^{-2} \cdot s^{-1}$)			
		75	150	300	600
Solenostemon scutellarioides	'Big Red Judy'	2.8 ± 0.2 Babc	4.2 ± 0.3 ABbc	6.0 ± 0.6 Ad	6.7 ± 0.7 Ac
	'Dark Star'	2.2 ± 0.1 Cabcde	3.3 ± 0.3 BCbcd	5.1 ± 0.3 ABd	7.5 ± 0.6 Ac
	'Dipt in Wine'	2.2 ± 0.4 Babcde	3.5 ± 0.5 Bbc	6.4 ± 1.2 Ad	7.5 ± 1.4 Ac
	'LifeLime'	0.2 ± 0.0 Aef	0.3 ± 0.0 Ae	0.3 ± 0.0 Aef	0.7 ± 0.1 Afg
	'Royal Glissade'	2.2 ± 0.3 Cabcd	5.3 ± 0.4 Bab	11.4 ± 1.1 Aa	10.5 ± 1.0 Ab
	'Sedona'	3.8 ± 0.3 Da	7.1 ± 0.6 Ca	11.0 ± 1.4 Ba	14.0 ± 2.3 Aa
	'Solar Red'	3.3 ± 0.5 Bab	4.1 ± 0.5 ABbc	6.5 ± 0.8 Ac	6.3 ± 0.6 Acd
	'Twist and Twirl' Green	0.4 ± 0.1 Bdef	0.4 ± 0.1 Be	0.7 ± 0.2 ABef	3.1 ± 1.4 Aef
	'Twist and Twirl' Red	3.5 ± 0.7 Bab	5.4 ± 2.1 Babc	9.1 ± 2.7 Ab	4.6 ± 0.6 Bde
	'Versa Burgundy to Green'	1.0 ± 0.1 Acdef	1.6 ± 0.4 Ade	2.8 ± 0.4 Ae	2.9 ± 0.7 Af
	'Versa Crimson Gold'	2.0 ± 0.3 Bbcdef	3.4 ± 0.5 Bcd	7.4 ± 1.1 Ad	3.9 ± 0.8 ABef
	'Versa Lime'	0.2 ± 0.0 Af	0.2 ± 0.0 Ae	0.3 ± 0.0 Af	0.4 ± 0.0 Ag
Pennisetum advena	-	0.3 ± 0.0 B	0.4 ± 0.0 B	0.4 ± 0.0 B	1.3 ± 0.2 B

[z] Irradiance was not significant in *Panicum virgatum*. Pooled across irradiance, 'Heavy Metal' (0.2 ± 0.0) had a lower anthocyanin ratio than Ruby Ribbons™ (2.4 ± 0.07).

Table C.8. Analysis of variance of five *Solenostemon scutellarioides* (coleus) and two *Panicum virgatum* (switchgrass) cultivars in response to temperature (Chapter 5). The experimental design was a three-way factorial (temperature, cultivar, and day) arranged in a split plot design, with temperature as the main plot, cultivar as the subplot, and repeated measures (day) on the subplot. Each species was analyzed separately.

Species	Source	df	Anthocyanin	% Anth	Total Chl	% Tot Chl	Chl *a*/*b*	Anth/Chl ratio
Solenostemon	Rep	2	0.0007	0.16	0.01	0.19	0.22	0.07
scutellarioides	Temperature	3	0.01	0.13	0.003	0.02	0.002	0.001
	Cultivar	4	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	T x C	12	<0.0001	<0.0001	0.30	<0.0001	<0.0001	<0.0001
	Day	5	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	T x D	15	<0.0001	0.0002	<0.0001	<0.0001	<0.0001	<0.0001
	C x D	20	<0.0001	<0.0001	<0.0001	<0.0001	0.0005	<0.0001
	T x C x D	60	<0.0001	<0.0001	0.001	0.0005	0.0008	<0.0001
Panicum	Rep	2	0.08	0.74	0.39	0.03	0.0008	0.25
virgatum	Temperature	3	0.29	0.36	0.27	0.41	0.14	0.50
	Cultivar	1	0.02	0.004	0.21	0.15	0.02	0.07
	T x C	3	0.55	0.43	0.45	0.34	0.09	0.49
	Day	5	0.003	<0.0001	0.63	0.32	<0.0001	0.07
	T x D	15	0.52	0.71	0.35	0.73	0.15	0.13
	C x D	5	<0.0001	<0.0001	0.44	0.48	0.55	0.08
	T x C x D	15	0.90	0.93	0.84	0.65	0.99	0.12

Table C.9. Analysis of variance of five *Solenostemon scutellarioides* (coleus) and two *Panicum virgatum* (switchgrass) cultivars in response to temperature after 25 d (Chapter 5). The experimental design was a two-way factorial (temperature, cultivar) arranged in a split plot design, with temperature as the main plot and cultivar as the subplot. Each species was analyzed separately.

Species	Source	df	Anthocyanin	% Anth	Total Chl	% Tot Chl	Chl *a*/*b*	Anth/Chl ratio
Solenostemon	Rep	2	<0.0001	0.003	0.0005	0.02	0.07	0.06
scutellarioides	Temperature	3	<0.0001	0.003	0.007	0.005	0.01	0.12
	Cultivar	4	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	<0.0001
	T x C	12	<0.0001	<0.0001	<0.0001	<0.0001	0.005	<0.0001
Panicum	Rep	2	0.24	0.81	0.49	0.0004	<0.0001	0.17
virgatum	Temperature	3	0.44	0.64	0.22	0.76	0.43	0.75
	Cultivar	1	<0.0001	<0.0001	0.53	0.10	0.20	0.01
	T x C	3	0.09	0.24	0.35	0.24	0.25	0.83

Table C.10. Total chlorophyll content ($\mu g \cdot cm^{-2}$) of five *Solenostemon scutellarioides* (coleus) cultivars in response to temperature (mean ± $_{SE}$, n=12) after a 25 d exposure. Means within each column followed by different uppercase letters or means within each row followed by different lowercase letters are significantly different using Tukey's HSD ($\alpha = 0.05$).

Temperature (°C)	Cultivar				
	'Big Red Judy'	'LifeLime'	'Royal Glissade'	'Twist and Twirl' green	'Twist and Twirl' red
12	4.5 ± 0.5 Bb	0.9 ± 0.2 Bb	2.7 ± 0.4 Ab	2.7 ± 0.5 Cb	15.0 ± 2.4 Ba
18	10.5 ± 0.8 ABb	6.5 ± 0.7 ABb	5.3 ± 0.6 Ab	11.3 ± 1.9 BCb	26.6 ± 2.9 Aa
24	12.2 ± 1.4 ABbc	8.2 ± 1.2 ABc	5.6 ± 0.6 Ac	15.5 ± 3.9 Bab	21.2 ± 3.5 ABa
30	18.9 ± 1.9 Ab	15.7 ± 2.1 Abc	11.4 ± 1.3 Ac	27.2 ± 4.4 Aa	27.1 ± 3.4 Aa

Table C.11. Anthocyanin/chlorophyll ratio of five *Solenostemon scutellarioides* (coleus) cultivars in response to temperature (mean ± SE, n=12) after a 25 d exposure. Means within each column followed by different uppercase letters or means within each row followed by different lowercase letters are significantly different using Tukey's HSD ($\alpha = 0.05$).

Temperature (°C)	Cultivar				
	'Big Red Judy'	'LifeLime'	'Royal Glissade'	'Twist and Twirl' green	'Twist and Twirl' red
12	27.7 ± 3.5 Aa	3.4 ± 1.5 Ab	20.5 ± 6.2 Aa	1.4 ± 0.5 Ab	7.1 ± 1.1 Ab
18	7.4 ± 0.7 Bab	0.4 ± 0.0 Ab	12.6 ± 1.9 ABa	0.5 ± 0.1 Ab	3.1 ± 0.4 Ab
24	6.2 ± 0.9 Bab	0.3 ± 0.0 Ab	11.1 ± 2.5 ABa	0.9 ± 0.5 Ab	3.5 ± 0.6 Aab
30	3.9 ± 0.4 Ba	0.3 ± 0.0 Aa	5.8 ± 0.8 Ba	0.3 ± 0.0 Aa	2.2 ± 0.3 Aa

Table C.12. Analysis of variance of three *Solenostemon scutellarioides* (coleus) and two *Panicum virgatum* (switchgrass) cultivars in response to temperature and irradiance (treatment) after 25 d (Chapter 5). The experimental design was a three-way factorial (temperature, irradiance, and cultivar) arranged in a split plot design, with temperature and irradiance as the main plot and cultivar as the subplot. Each species was analyzed separately.

Source	Species			
	Solenostemon scutellarioides		*Panicum virgatum*	
	df	*P*-value	df	*P*-value
Rep	2	0.02	2	0.09
Temperature	2	0.26	2	0.24
Irradiance	1	0.0008	1	0.02
T x I	2	0.78	2	0.44
Cultivar	2	<0.0001	1	<0.0001
C x T	4	<0.0001	2	0.03
C x I	2	0.0002	1	0.29
C x T x I	4	0.10	2	0.68