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Stem girdling manipulates leaf sugar concentrations and anthocyanin expression in sugar maple trees during autumn

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Summary To better understand the effects of sugar accumulation on red color development of foliage during autumn, we compared carbohydrate concentration, anthocyanin expression and xylem pressure potential of foliage on girdled versus non-girled (control) branches of 12 mature, open-grown sugar maple (*Acer saccharum* Marsh.) trees. Half of the study trees were known to exhibit mostly yellow foliar coloration and half historically displayed red coloration. Leaves from both girdled and control branches were harvested at peak color expression (i.e., little or no chlorophyll present). Disruption of phloem export by girdling increased foliar sucrose, glucose and fructose concentrations regardless of historical tree color patterns. Branch girdling also increased foliar anthocyanin expression from 50.4 to 66.7% in historically red trees and from 11.7 to 54.2% in historically yellow trees, the latter representing about a fivefold increase compared with control branches. Correlation analyses indicated a strong and consistent relationship between foliar red coloration and sugar concentrations, particularly glucose and fructose, in both girdled and control branches. Measures of xylem pressure potentials confirmed that girdling was a phloem-specific treatment and had no effect on water transport to distal leaves. Results indicate that stem girdling increased foliar sugar concentrations and enhanced anthocyanin expression during autumn in sugar maple foliage. Native environmental stresses (e.g., low autumn temperatures) that reduce phloem transport may promote similar physiological outcomes.

Keywords: Acer saccharum, carbohydrates, phloem transport, pigments, senescence.

Introduction

The biology of autumnal chlorophyll catabolism is well understood (Buchanan-Wollaston 1997, Matile 2000, Thomas et al. 2001). Decreasing photoperiods and temperatures are major contributors to the seasonal conversion of tree canopy color from green to yellow in temperate deciduous forests. Another prominent change in leaf pigmentation is the de novo synthesis of anthocyanins that occurs in some deciduous hardwood species such as sugar maple (*Acer saccharum* Marsh.), and produces brilliant autumnal displays of red and purple leaves at northern latitudes. Anthocyanins, which are responsible for the red coloration, are synthesized by the flavanoid pathway and reside mainly in the vacuoles of foliar mesophyll and epidermal cells of angiosperms (Chalker-Scott 1999, Lee and Gould 2002). They are also found in flowers, fruits, stems and even roots of many flowering species (Lee and Gould 2002). In temperate regions, biosynthesis of foliar anthocyanins is most commonly associated with foliar senescence in autumn (Ishikura 1972, Chang et al. 1989, Feild et al. 2001, Hoch et al. 2001, Lee et al. 2002, Schaberg et al. 2003); however, its presence has also been reported in springtime juvenile foliage of deciduous species such as those of the family Aceraceae (Ji et al. 1992). Numerous studies have linked environmental stress factors, such as drought, nutrient deficiency, wounding, pathogen infection, ozone, high and low temperatures, and ultraviolet light exposure to anthocyanin biosynthesis (Chalker-Scott 1999, 2002, Hoch et al. 2001, Rostás et al. 2002, Steyn et al. 2002, Close and Beadle 2003). Because anthocyanin expression often follows exposures to stress, many hypotheses suggesting potential benefits of anthocyanins in supporting adaptive stress responses have been proposed (see review by Lee and Gould 2002).

Foliar sugar accumulation has also been documented to increase following exposure to a range of environmental stresses such as ozone (Einig et al. 1997, Topa et al. 2001), low temperatures (Guo and Oosterhuis 1995, Strand et al. 1997, Sasaki et al. 2001), elevated carbon dioxide concentration (Barnes et al. 1995) and insect damage (Andersen et al. 2002). Examples of foliar carbohydrate accumulation as a result of physiological stress are well represented by studies involving experimentally induced manipulations of source–sink relationships by mechanical girdling in crops such as spinach (Krapp and Stitt 1995), soybean (Ohyama and Kawai 1983), maize (Jeanette et al. 2000) and potato (Schulz et al. 1998). Similarly, trunk or shoot girdling of fruit trees such as alternate-bearing 'Murcott' (a *Citrus* reticulate hybrid) (Li et al. 2003) and apple (*Malus sylvestris* (L.) Mill var. *domestica* (Borkh.)) (Zhou and Quebedeaux 2003) resulted in increases in foliar carbohydrates. The co-occurrence of anthocyanin expression and sugar enhancement has been found in laboratory studies on food

crops such as strawberry (*Fragaria ananassa* Duchesne ex Rozier), grape (*Vitis labruscana* Bailey), radish (*Raphanus sativus* L.) and Indian almond (*Terminalia catappa* L.) (Dube et al. 1993, Mori and Sakurai 1994, Hiratsuka et al. 2001, Hara et al. 2003), and in foliage of *Arabidopsis thaliana* (L.) Heynh. seedlings (Teng et al. 2005) and maize plants (Jeannette et al. 2000). Although not as well understood, field studies of temperate forest tree species have shown a similar relationship between increases in sugar concentration and enhanced anthocyanin expression, particularly during autumnal senescence in *Acer* (Ishikura 1972, Schaberg et al. 2003) and quaking aspen (*Populus tremuloides* Michx.) (Chang et al. 1989). We have obtained preliminary evidence that carbohydrates, especially sugars, may play an important role in triggering anthocyanin biosynthesis and in providing the necessary building blocks for its synthesis in senescing foliage of sugar maple (Schaberg et al. 2003). Sugars have also recently been shown to regulate the expression of genes involved in several plant metabolic functions including anthocyanin expression in vegetative tissues (Dube et al. 1993, Mori and Sakurai 1994, Hiratsuka et al. 2001, Hara et al. 2003).

Stem girdling has been used experimentally to test the relationship between foliar sugar accumulation and anthocyanin expression in maize (Jeanette et al. 2000). However, to our knowledge no similar test has been reported for a forest tree species. Here we present the results of an experiment in which we increased sugar concentrations of sugar maple leaves by branch girdling and phloem disruption, and evaluated the effects of this manipulation on anthocyanin expression. To determine if historic patterns of foliar pigmentation could be altered by controlling sugar availability, manipulations were conducted on branches from (1) trees with leaves that historically exhibited little anthocyanin production, and (2) trees with leaves that consistently exhibited high anthocyanin production. Information on the role(s) sugars play in promoting anthocyanin expression may provide the enhanced mechanistic understanding needed to evaluate the various hypothetical functions of anthocyanin production in leaves of temperate deciduous trees during autumn.

Materials and methods

Girdling and collection of plant material

Twelve open-grown sugar maple trees at the USDA Forest Service Northern Research Station in South Burlington, Vermont, USA (elevation 92 m) were selected from a pool of 80 trees based on their consistent history of either red or yellow autumnal leaf coloration. The selected trees were about 10 m tall and 25 years old, and were scattered throughout the site. Each October, beginning in 1998, at peak color in the Champlain Valley of Vermont, the crowns of the selected trees were visually assessed for their percent red and yellow coloration of leaves within tree crowns. Analysis of variance of data for the years before the start of the current experiment showed significant differences in percent red and yellow crown coloration among trees ($P < 0.0001$). Based on this analysis, six of the twelve study trees were designated as being "historically red" (28.6 ± 3.8 mean percent red in crowns for the three years before this study), whereas the other six trees were considered "historically yellow" (only 3.9 ± 1.4 mean percent red in crowns for the same period). Mean percent yellow coloration during the same time period was 35.8 ± 5.8% for historically red trees and 70.3 ± 4.6% for historically yellow trees. Trees with red and yellow color histories were selected to fully represent the variability in autumnal color development in sugar maple trees in Vermont.

On August 24, 2000, two south-facing, sunlit branches from the lower third of the crown were girdled and flagged on each tree. Girdling was completed by carefully removing the outer bark and phloem tissue from the branch with a standard razor blade. The width of the girdle was about 2 cm. Parafilm was tightly wrapped around the wound to protect the girdled area from pathogen invasion. Two non-girdled control branches (one adjacent to each of the two girdled stems), also on the south side of the trees, were flagged per tree. This process was repeated on September 9, 2000 with a separate set of branches on the same 12 trees. When leaves of girdled and control branches were visually determined to have reached peak color, they were harvested and subsequent color and carbohydrate analyses were performed in the laboratory. Peak color was estimated as the time when leaves exhibited little or no chlorophyll expression, displayed a mosaic of yellow or red coloration, or both, and showed no visible signs of necrosis. Leaves were collected as early as September 15 and collections continued until October 20.

Carbohydrate analysis

Concentrations of the soluble sugars glucose, fructose and sucrose were determined in foliar ethanol extracts as described by Hinesley et al. (1992). Chlorophyll was removed from the soluble sugars using a C_{18} Sep-Pak Plus Cartridge (Waters Corp., Milford, MA). A subsample of the extract was dried at 37 °C in a limited insert vial, reconstituted in 200 µl of 0.1 mM Ca EDTA and filtered through a 0.45 µm syringe filter. Filtrates were analyzed by high performance liquid chromatography (Waters Corp.) through a Sugar-Pak column at 90 °C and with 0.1 mm Ca EDTA as the solvent at a flow rate of 0.6 ml min^{-1}. Waters Millenium 2000 software was used to quantify sugar concentrations, which were expressed as mg cm^{-2} leaf area. The pellet from the ethanol extract was gelatinized with 0.2 M KOH, boiled for 30 min and neutralized with 1 M acetic acid. The solubilized starch was hydrolyzed to glucose by incubating with amyloglucosidase (10115, Sigma Chemicals) in 0.1 M acetate buffer (pH 4.5) at 55 °C for 30 min. The reaction was terminated by boiling for 4 min, after which the supernatant was centrifuged at 1000 *g* for 10 min. Starch was quantified as glucose equivalents (glucose assay 115-A, Sigma Chemicals) as described by Hendrix (1993). Absorbances of samples and glucose standards were measured at 492 nm with an Inter-Med TIM-200 ELISA plate reader. Final starch concentrations were determined with reference to glucose standard curves and expressed as µg cm^{-2} leaf area.

Xylem pressure potential

To evaluate whether girdling was a phloem-specific treatment or whether it also reduced xylem recharge potential, predawn and midday xylem pressure potentials (MPa) were measured in mid September as described by Ritchie and Hinckley (1975) on leaves of both girdled and control branches with a PMS Model 1001 portable pressure chamber (PMS Instrument, Corvallis, OR).

Foliar color analysis

Red and yellow foliar coloration was measured by digital image analysis as described by Murakami et al. 2005. Leaves were scanned with an Epson Perfection 1200U flatbed color scanner (Epson America, Torrance, CA) at 240 dpi and images were acquired with Photoshop software (Ver. 5.0, Adobe Systems, San Jose, CA). Images were saved in tag image file format and imported into an NIH Image software program (U.S. National Institute of Health; http://rsb.info.nih.gov/nih-image) where percent red and yellow leaf areas were calculated. At the time of this study, resources were not available for direct analysis of pigment chemistry. However, the authors have since confirmed that the digital image analysis accurately represented both visual and chemical estimates of foliar color categories and pigment concentrations of sugar maple foliage. Specifically, in a separate study with 326 sugar maple leaves, percent red coloration and anthocyanin concentration were found to be significantly and positively related ($r^2 = 0.83$; $P < 0.0001$) (Murakami et al. 2005). It is well established that, with the exception of the winter accumulation of red carotenoids in some conifer shoots and evergreen flowering plants, most red leaves result from de novo synthesis of anthocyanins (Feild et al. 2001).

Statistical analyses

Student's *t*-tests were used to compare foliar xylem pressure potentials between girdled and control branches and to determine differences in percent color and carbohydrate concentrations between leaves of historically red and historically yellow trees. Differences in percent foliar color and carbohydrate concentrations between girdled and control branches were analyzed by paired *t*-tests. Relationships between foliar color and carbohydrate concentrations were evaluated by correlation analyses. Differences were considered statistically significant if $P \leq 0.05$. Because the statistical analyses revealed no difference in foliar color development and carbohydrate concentrations between trees girdled on August 24 and September 9, data from these dates were combined for subsequent analyses.

Results

Girdling as a phloem-specific disruption

Disruption of phloem transport by girdling consistently resulted in measurable increases in foliar sugar concentrations. Regardless of tree color history, sucrose, glucose and fructose concentrations ($P \leq 0.0001$) were significantly higher in leaves on girdled branches than in leaves on non-girdled control branches (Figure 1). Leaf sugar concentrations also differed significantly within individual tree color histories. In historically red trees, foliar sucrose ($P = 0.0235$), glucose ($P = 0.0140$) and fructose ($P \leq 0.0001$) concentrations were significantly higher in girdled branches than in control branches. Similarly, in historically yellow trees, foliar sucrose ($P = 0.0023$), glucose ($P = 0.0006$) and fructose ($P \leq 0.0001$) concentrations were significantly higher in girdled branches than in control branches, which contained little or no glucose and fructose in their leaves. Differences in foliar sugar concentrations between color history groups also existed, but only for girdled branches. Girdled, historically red trees had significantly higher foliar glucose concentrations than girdled, historically yellow trees ($P = 0.0385$). Control branches showed no difference in glucose concentrations between historically red and historically yellow trees ($P = 0.1827$). In contrast to the sugar data, foliar starch concentration did not differ significantly between all girdled (18.7 ± 4.3 μg cm^{-2}) and all control (18.4 ± 7.0 μg cm^{-2}) branches ($P = 0.9573$), nor was there a significant difference within individual historical color categories (data not shown).

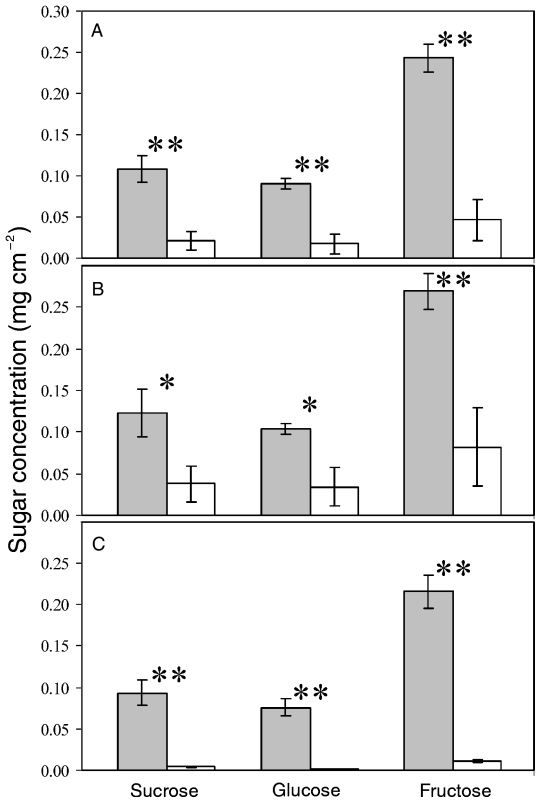

Figure 1. Mean sucrose, glucose and fructose concentrations in leaves of girdled (gray bars) and control (white bars) branches of (A) all *Acer saccharum* trees, (B) historically red trees and (C) historically yellow trees at time of peak color. Standard error bars represent ± 1 SE. Significant treatment differences are indicated as: **, $P < 0.01$; and *, $P < 0.05$.

Although girdling disrupted phloem transport, there was no evidence that this treatment altered water flow to leaves via the xylem. Foliar measurements of predawn (P = 0.6432) and midday (P = 0.5466) xylem pressure potentials were statistically indistinguishable between treatments, indicating that girdling did not alter the capacity of leaves distal to the girdle to recover from transpirational water losses (Figure 2). Thus, any treatment differences in leaf color expression and biochemistry were likely not caused by treatment-induced alterations in water relations.

Treatment effects on leaf color

Paired *t*-tests showed that, regardless of color history, foliar red coloration was significantly greater in girdled branches compared with control branches (P = 0.0008, Figure 3). Leaves of historically red, girdled branches exhibited 66.7% red coloration, whereas leaves of control branches expressed 50.4% red coloration (P = 0.0404). Yellow coloration of foliage from the same historically red trees was lower in girdled branches than in control branches (18.2 versus 36.0%; P = 0.0078). Girdling resulted in a significant and remarkably large increase in red coloration of foliage in historically yellow trees. Leaves of historically yellow control branches exhibited 11.7% red coloration and girdling increased this percentage to 54.2%, an almost fivefold increase over control values and a 14-fold increase over the 3-year mean of 3.9% of red coloration in historically yellow trees. Thus, enhanced anthocyanin production as a result of phloem disruption can occur above expected values even in trees historically known to express little red coloration in their crowns.

Correlation analyses highlighted a strong and consistent association between foliar red coloration and leaf sugar concentration. For example, we found significant positive relationships between percent red coloration and glucose (r = 0.78; P = 0.0043) and fructose (r = 0.80; P = 0.0031) concentrations in leaves of girdled branches (Table 1). This was especially evident in leaves of girdled historically yellow trees that expressed an unusually high percentage of red coloration (54.2%)

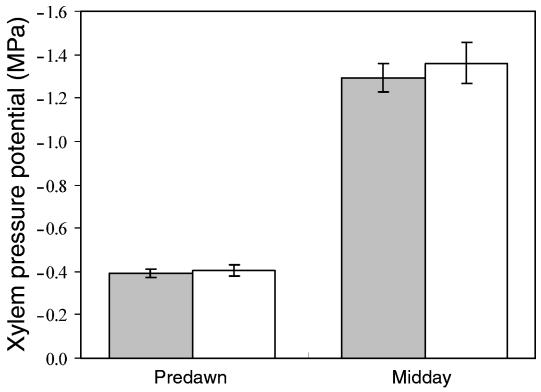

Figure 2. Mean predawn and midday foliar xylem pressure potentials for girdled (gray bars) and control (white bars) branches of *Acer saccharum* trees. Standard error bars represent ± 1 SE.

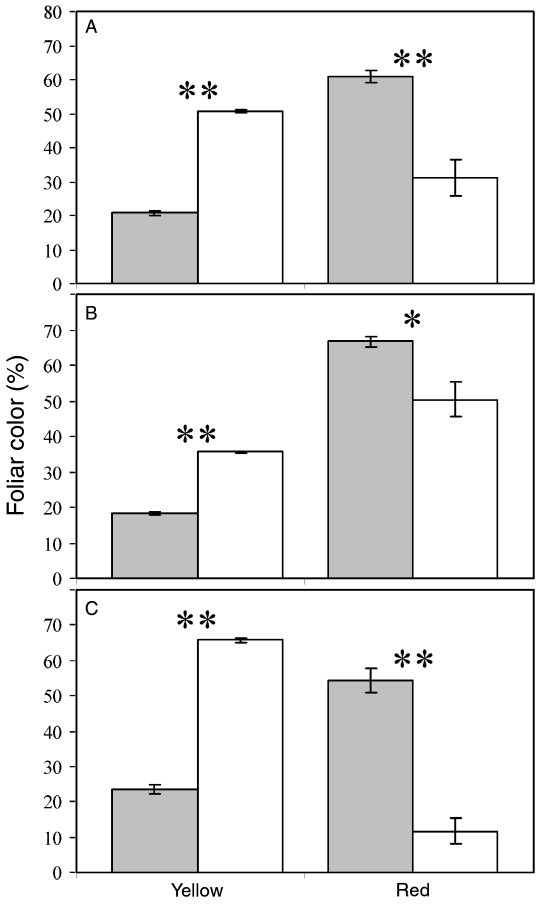

Figure 3. Percentage of foliar leaf color for girdled (gray bars) and control (white bars) branches of (A) all *Acer saccharum* trees, (B) historically red trees and (C) historically yellow trees. Standard error bars represent ± 1 SE. Significant treatment differences are indicated as: **, $P < 0.01$; and *, $P < 0.05$.

Table 1. Correlation coefficients (r) and P values for percent foliar color versus carbohydrate concentration in girdled and control branches of twelve mature *Acer saccharum* trees. Relationships are considered significant when $P < 0.05$.

Color (%)	Sugar (mg g^{-1})	r	P
Girdled			
Yellow	Glucose	–0.76	0.0064
	Fructose	–0.65	0.0316
Red	Glucose	0.78	0.0043
	Fructose	0.80	0.0031
Control			
Yellow	Sucrose	–0.56	0.0605
	Glucose	–0.58	0.0479
	Fructose	–0.59	0.0442
Red	Sucrose	0.62	0.0306
	Glucose	0.63	0.0281
	Fructose	0.65	0.0209

compared with their previously documented 3-year mean of 3.9% in naturally senescing leaves. Red coloration of leaves from control branches had similar positive and significant relationships with glucose ($r = 0.63$; $P = 0.0281$) and fructose ($r = 0.65$; $P = 0.0209$) concentrations as the foliage from girdled branches, but with slightly lower correlation coefficients. In contrast to the positive relationships found for sugars and the expression of red leaf pigmentation, significant negative relationships existed between percent yellow coloration and foliar glucose ($r = -0.76$; $P = 0.0064$) and fructose ($r = -0.65$; $P = 0.0316$) concentrations for girdled branches. In control branches, as in their girdled counterparts, percent yellow and sugar concentrations were both significantly and negatively correlated with glucose ($r = -0.58$; $P = 0.0479$) and fructose ($r = -0.58$; $P = 0.0442$).

Overall, phloem disruption by girdling caused an increase in foliar sugar accumulation (mainly glucose and fructose) and an increase in anthocyanin expression relative to the experimental controls. Combined with experimental evidence for other species, particularly *Arabidopsis* and some agricultural species, the treatment-induced effects we have documented may help elucidate the physiological function of anthocyanins in leaves of sugar maple trees during autumn.

Discussion

Accumulation of sugars as signals of anthocyanin expression

As expected, phloem disruption of branches of sugar maple trees in late summer caused the accumulation of assimilates such as sucrose, glucose and fructose in foliage during autumnal senescence (Figure 1). In addition to increased foliar sugar concentrations, girdling treatments simultaneously enhanced anthocyanin expression in sugar maple leaves (Figure 3). Enhanced anthocyanin expression was particularly evident in girdled historically yellow trees for which girdling-induced sugar accumulation was associated with patterns of color expression that were inconsistent with historical measurements and with those from non-girdled tissues (Figure 3). The successful manipulation of anthocyanin expression in historically yellow trees to resemble that of historically red trees highlights the inducible nature of this trait, and is consistent with the likelihood that sugar accumulation influences the biochemistry of this process. Previous laboratory studies with food crops and *Arabidopsis*, and limited field evaluations of temperate forest species during autumnal senescence have circumstantially linked increases in sugar concentrations and enhanced anthocyanin expression. In addition to correlative evidence, sugars have been shown to induce transcription of genes required for anthocyanin biosynthesis in several herbaceous plant species. In radish hypocotyls, activation of the anthocyanin biosynthetic genes encoding chalcone synthase and anthocyanidin synthase was accompanied by the accumulation of anthocyanin during sucrose treatments (Hara et al. 2003). Likewise, a sucrose-specific pathway was found to signal activation of the anthocyanin biosynthetic gene, *PAP1*, in *Arabidopsis* (Solfanelli et al. 2006). Although foliar glucose and fructose concentrations were directly related to increases in anthocyanin biosynthesis in girdled branches of sugar maple, we also found a significant relationship between foliar sucrose concentration and enhanced red expression in control branches (Table 1). Additional studies that control for the concentration and composition of sugars available to senescing (e.g., exposed to cold and high light) or environmentally stressed (e.g., exposed to high temperature, UV, insect or fungal pathogens) sugar maple foliage would further elucidate the connections between stress exposure, tissue sugar accumulations and anthocyanin formation.

Sugars and anthocyanins as possible integrators of stress response in sugar maple leaves

Our findings highlight the influence of increased foliar sugar concentrations on the autumn expression of senescence and anthocyanin in experimentally manipulated sugar maple shoots. However, the same basic physiological connections associated with experimental manipulation in our study are likely also pertinent to reports of increased red leaf coloration coincident with wounding (Jeanette et al. 2000) and certain types of insect and fungal damage (Costa-Arbulú et al. 2001, Rostás et al. 2002) in which disruptions of phloem transport and sugar export also occur. Low temperatures during autumn lead to reductions in phloem transport (Keskitalo et al. 2005), which would be expected to increase foliar sugar concentrations and enhance anthocyanin expression on a broader spatial scale than localized wounding or insect injury—perhaps accounting for changes in patterns of landscape-level foliar displays.

Additionally, low temperatures result in the down-regulation of photosynthesis, which when accompanied by high irradiances can induce photooxidative damage (Pietrini et al. 2002, Keskitalo et al. 2005). It has been shown that low temperatures induce the breakdown of chlorophyll and subsequently generate oxidative activity (Lee et al. 2002). Thus, during leaf senescence, anthocyanins may protect leaves from the negative effects of photoinhibition by scavenging reactive oxygen species produced by photooxidation during periods of low temperature (Huner et al. 1998). Gould et al. (2002) presented supporting evidence in *Pseudowintera colorata* (Raoul) Dandy showing that anthocyanins are effective scavengers of hydrogen peroxide in vivo. Other evaluations of antioxidant activity in red leaves have led to a better understanding of its protective role in vitro (Neill et al. 2002, Neill and Gould 2003).

In addition to the roles of anthocyanins in photo-protection and as antioxidants, foliar expression of anthocyanins may allow for a prolonged period of nutrient resorption especially in trees whose leaf period may be inherently short because of a combination of environmental stresses (Hoch et al. 2001, 2003, Feild et al. 2001, Ougham et al. 2005). Hoch et al. (2003) proposed a resorption protection hypothesis in which anthocyanins of senescing foliage shade the photosynthetic system and prevent photoinhibition, thereby allowing for enhanced resorption of nutrients, particularly nitrogen. This extended period of nutrient resorption is especially evident in

plants that experience low temperatures seasonally (Hoch et al. 2001). Similarly, Feild et al. (2001) emphasized the dual role of anthocyanins in senescing foliage of red osier dogwood (*Cornus stolonifera* Michx.) as scavengers of reactive oxygen species and facilitators of nutrient recovery. A recent study by Schaberg et al. (2008) may provide the first anatomical evidence in support of the nutrient resorption hypothesis of anthocyanin function. Microscopic evaluations of senescing red and green leaves of sugar maple showed incomplete formation of the abscission layer through the petiole vasculature, whereas formation of the abscission layer in petioles of yellow leaves was complete, indicating the termination of phloem transport. These results demonstrate a relationship between foliar coloration and abscission zone formation and suggest that, at least during senescence, red expression in sugar maple may allow for an extended period of nutrient and sugar translocation compared with that in yellow leaf counterparts.

In conclusion, the benefits of enhanced anthocyanin production during autumnal senescence allowing for greater nutrient absorption may be particularly important to sugar maple trees because recent evidence suggests that nutrient and carbohydrate stores in this species may be marginal compared with the amounts needed to support long-term health. Disruptions in nutrition and carbon relations are thought to be fundamental factors contributing to the decline of sugar maple in the northeastern USA and adjacent Canada (Horsley et al. 2000, Hallett et al. 2006). The extreme sensitivity of sugar maple to decline associated with these disruptions relative to other species on the same sites provides an indication that sugar maple trees have unique vulnerabilities or requirements regarding energy and nutrition pathways. Our research and related studies describe an apparent sequence of events that enable environmentally stressed or senescing deciduous trees to maximize their ability for carbohydrate and nutrient resorption.

We propose that environmental stresses, such as low temperature, initiate a chain of events leading to increases in foliar sugars and enhanced anthocyanin expression, which in turn may allow for extended opportunities to retrieve and transport nutrient and carbon gains to winter storage sites. Specifically for sugar maple, this mechanism may augment the winter storage of essential constituents that help prevent health declines. Future comparisons between summer and autumn carbohydrate, nutrient and pigment concentrations would further clarify this relationship. Sugar maple's distinctive ability to produce both red and yellow foliar coloration provides a unique platform for examining the physiological role of anthocyanin expression during environmental stress and natural senescence processes.

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References

Andersen, P.C., B.V. Brodbeck and D.C. Herzog. 2002. Girdling-induced nutrient accumulation in aboveground tissue of peanuts and subsequent feeding by *Spissistilus festinus*, the three-cornered alfalfa hopper. Entomol. Exp. Appl. 103:139–149.

Barnes, J.D., T. Pfirrmann, K. Steiner, C. Lutz, U. Busch, H. Kuchenhoff and H.D. Payer. 1995. Effects of elevated CO_2 elevated O_3 and potassium deficiency on Norway spruce [*Picea abies* (L.) Karst.]: seasonal changes in photosynthesis and nonstructural carbohydrate content. Plant Cell Environ. 18:1345–1357.

Buchanan-Wollaston, V. 1997. The molecular biology of leaf senescence. J. Exp. Bot. 48:181–199.

Chalker-Scott, L. 1999. Environmental significance of anthocyanins in plant stress responses. Photochem. Photobiol. 70:1–9.

Chalker-Scott, L. 2002. Do anthocyanins function as osmoregulators in leaf tissues? *In* Anthocyanins in Leaves. Eds. K.S. Gould and D.W. Lee. Advances in Botanical Research, Academic Press, London, pp 103–127.

Chang, K.G., G.H. Fechner and H.A. Schroeder. 1989. Anthocyanins in autumn leaves of quaking aspen in Colorado. For. Sci. 35: 229–236.

Close, D.C. and C.L. Beadle. 2003. The ecophysiology of foliar anthocyanin. Bot. Rev. 69:149–161.

Costa-Arbulú, C., E. Gianoli, W.L. Gonzáles and H.M. Niemeyer. 2001. Feeding by the aphid *Sipha flava* produces a reddish spot on leaves of *Sorghum halepense*: an induced defense? J. Chem. Ecol. 27:273–283.

Dube, A., S. Bharti and M.M. Laloraya. 1993. Inhibition of anthocyanin synthesis and phenylalanine ammonialyase activity by Co^{2+} in leaf disks of *Terminalia catappa*. Physiol. Plant. 88:237–242.

Einig, W., U. Lauxmann, B. Hauch, R. Hampp, W. Landolt, S. Maurer and R. Matyssek. 1997. Ozone-induced accumulation of carbohydrates changes enzyme activities of carbohydrate metabolism in birch leaves. New Phytol. 137:673–680.

Feild, T.S., D.W. Lee and N.M. Holbrook. 2001. Why leaves turn red in autumn. The role of anthocyanins in senescing leaves of red-osier dogwood. Plant Physiol. 127:566–574.

Gould, K.S., J. McKelvie and K.R. Markham. 2002. Do anthocyanins function as antioxidants in leaves? Imaging of H_2O_2 in red and green leaves after mechanical injury. Plant Cell Environ. 25: 1261–1269.

Guo, C.X. and D.M. Oosterhuis. 1995. Pinitol occurrence in soybean plants as affected by temperature and plant growth regulators. J. Exp. Bot. 46:249–253.

Hallett, R.A., S.W. Bailey, S.B. Horsley and R.P. Long. 2006. Influence of nutrition and stress on sugar maple at a regional scale. Can. J. For. Res. 36:2235–2246.

Hara, M., K. Oki, K. Hoshino and T. Kuboi. 2003. Enhancement of anthocyanin biosynthesis by sugar in radish (*Raphanus sativus*) hypocotyls. Plant Sci. 164:259–265.

Hendrix, D.L. 1993. Rapid extraction and analysis of nonstructural carbohydrates in plant tissue. Crop Sci. 33:1306–1311.

Hinesley, S.B., D.M. Pharr, L.K. Snelling and S.R. Funderbunk. 1992. Foliar raffinose and sucrose in four conifer species: relationships with seasonal temperature. J. Am. Soc. Hortic. Sci. 117: 852–855.

Hiratsuka, S., H. Onodera, Y. Kawai, T. Kubo, H. Itoh and R. Wada. 2001. ABA and sugar effects on anthocyanin formation in grape berry cultured in vitro. Sci. Hortic. 90:121–130.

Hoch, W.A., E.L. Zeldin and B.H. McCown. 2001. Physiological significance of anthocyanins during autumnal leaf senescence. Tree Physiol. 21:1–8.

Hoch, W.A., E.L. Singsaas and B.H. McCown. 2003. Resorption protection. Anthocyanins facilitate nutrient recovery in autumn by shielding leaves from potentially damaging light levels. Plant Physiol. 133:1296–1305.

Horsley, S.B., R.P. Long, S.W. Bailey, R.A. Hallett and T.J. Hall. 2000. Factors associated with the decline disease of sugar maple on the Allegheny Plateau. Can. J. For. Res. 30:1365–1378.

Huner, P.A., G. Oquist and F. Sarhan. 1998. Energy balance and acclimation to light and cold. Trends Plant Sci. 3:224–230.

Ishikura, N. 1972. Anthocyanins and other phenolics in autumn leaves. Phytochemistry 11:2555–2558.

Jeanette, E., A. Reyss, N. Gregory, P. Gantet and J.-L. Prioul. 2000. Carbohydrate metabolism in a heat-girdled maize source leaf. Plant Cell Environ. 23:61–69.

Ji, S.-B., M. Yokoi, N. Saito and L.-S. Mao. 1992. Distribution of anthocyanins in Aceraceae leaves. Biochem. Syst. Ecol. 20: 771–781.

Keskitalo, J., G. Bergquist, P. Gardeström and S. Jansson. 2005. A cellular timetable of autumn senescence. Plant Physiol. 139: 1635–1648.

Krapp, A. and M. Stitt. 1995. An evaluation of direct and indirect mechanisms for the "sink-regulation" of photosynthesis in spinach: changes in gas exchange, carbohydrates, metabolites, enzyme activities and steady-state transcript levels after cold-girdling source leaves. Planta 195:313–323.

Lee, D.W. and K.S. Gould. 2002. Anthocyanins in leaves and other vegetative organs: an introduction. *In* Anthocyanin in Leaves. Eds. K.S. Gould and D.W. Lee. Advances in Botanical Research, Academic Press, London, pp 1–16.

Lee, D.W, J. O'Keefe, N.M. Holbrook and T.S. Feild. 2002. Pigment dynamics and autumn leaf senescence in a New England deciduous forest, eastern USA. Ecol. Res. 18:677–694.

Li, C.-Y., D. Weiss and E.E. Goldschmidt. 2003. Girdling affects carbohydrate-related gene expression in leaves, bark and roots of alternate-bearing citrus trees. Ann. Bot. 92:137–143.

Matile, P. 2000. Biochemistry of Indian summer: physiology of autumnal leaf coloration. Exp. Gerentol. 35:135–158.

Mori, T. and M. Sakurai. 1994. Production of anthocyanin from strawberry cell suspension cultures; effects of sugar and nitrogen. J. Food Sci. 59:588–593.

Murakami, P.F., M.R. Turner, A.K. van den Berg and P.G. Schaberg. 2005. An instructional guide for leaf color analysis using digital imaging software. Gen. Tech. Rep. NE-327, USDA, For. Serv., Northeastern Res. Stn., 33 p.

Neill, S.O. and K.S. Gould. 2003. Anthocyanins in leaves: light attenuators or antioxidants? Funct. Plant Biol. 30:865–873.

Neill, S.O., K.S. Gould, P.A. Kilmartin, K.A. Mitchell and K.R. Markham. 2002. Antioxidant activities of red versus green leaves in *Elatostema rugosum*. Plant Cell Environ. 25:539–547.

Ohyama, T. and S. Kawai. 1983. Nitrogen assimilation and transport in soybean leaves: investigation by petiole girdling treatment. Soil Sci. Plant Nutr. 29:227–231.

Ougham, H.J., P. Morris, and H. Thomas. 2005. The colors of autumn leaves as symptoms of cellular recycling and defenses against environmental stresses. Curr. Top. Dev. Biol. 66:135–160.

Pietrini, F., M.A. Iannelli and A. Massacci. 2002. Anthocyanin accumulation in the illuminated surface of maize leaves enhances protection from photo-inhibitory risks at low temperature, without further limitation to photosynthesis. Plant Cell Environ. 25: 1251–1259.

Ritchie, G.A. and T.M. Hinckley. 1975. The pressure chamber as an instrument of ecological research. Adv. Ecol. Res. 9:165–254.

Rostás, M., R. Bennett and M. Hilker. 2002. Comparative physiological responses in Chinese cabbage induced by herbivory and fungal infection. J. Chem. Ecol. 28:2449–2463.

Sasaki, H., K. Ichimura, S. Imada and S. Yamaki. 2001. Sucrose synthase and sucrose phosphate synthase, but not acid invertase, are regulated by cold acclimation and deacclimation in cabbage seedlings. J. Plant Physiol. 158:847–852.

Schaberg, P.G., A.K. van den Berg, P.F. Murakami, J.B. Shane and J.R. Donnelly. 2003. Factors influencing red expression in autumn foliage of sugar maple trees. Tree Physiol. 23:325–333.

Schaberg, P.G., P.F. Murakami, M.R. Turner, H.K. Heitz and G.J. Hawley. 2008. Association of red coloration with senescence of sugar maple leaves in autumn. Trees doi:10.1007/s00468-008-0217-8.

Schulz, A., C. Kühn, J.W. Riesmeier and W.B. Frommer. 1998. Ultrastructural effects in potato leaves due to antisense-inhibition of the sucrose transporter indicate an apoplasmic mode of phloem loading. Planta 206:533–543.

Solfanelli, C., A. Poggi, E. Loreti, A. Alpi and P. Perata. 2006. Sucrose-specific induction of the anthocyanin biosynthetic pathway in *Arabidopsis*. Plant Physiol. 140:637–646.

Steyn, W.J., S.J.E. Wand, D.M. Holcroft and G. Jacobs. 2002. Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. New Phytol. 155:349–361.

Strand, A., V. Hurry, P. Gustafsson and P. Gardestrom. 1997. Development of *Arabidopsis thaliana* leaves at low temperatures releases the suppression of photosynthesis and photosynthetic gene expression despite the accumulation of soluble carbohydrates. Plant J. 12:605–614.

Teng, S., J. Keurentjes, L. Bentsink, M. Koornneef and S. Smeekens. 2005. Sucrose-specific induction of anthocyanin biosynthesis in *Arabidopsis* requires the *MYB75/PAP1* gene. Plant Physiol. 139: 1840–1852.

Thomas, H., H. Ougham and S. Hörtensteiner. 2001. Recent advances in the cell biology of chlorophyll catabolism. Adv. Bot. Res. 35: 1–52.

Topa, M., D. Vanderklein and A. Corbin. 2001. Effects of elevated ozone and low light on diurnal and seasonal carbon gains in sugar maple. Plant Cell Environ. 24:663–677.

Zhou, R. and B. Quebedeaux. 2003. Changes in photosynthesis and carbohydrate metabolism in mature apple leaves in response to whole plant source–sink manipulation. J. Am. Soc. Hortic. Sci. 128:113–119.