

Anthocyanins in Autumn Leaf Senescence

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ABSTRACT

Anthocyanins are synthesized during leaf senescence in certain plants across virtually all biomes, but are most spectacular in the autumn foliage of temperate deciduous forests. The patterns of color production in senescing foliage depend at least partly upon species composition and their phenology. Both ecological and physiological explanations have been raised to explain why plants produce this pigment just before leaf fall. Physiological explanations, as photoprotection, predict that cyanic leaves would be better able to resorb nitrogen during the process of chlorophyll degradation. Ecological explanations predict better dispersal of propagules advertised by association with the brilliantly colored leaves (plausible for only a minority of species), or warning against egg-laying activity of herbivorous insects, as aphids. These hypotheses make predictions that we now can test, to help us understand this old mystery – and majestic phenomenon.

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I. INTRODUCTION

This volume explores the wide variety of circumstances under which anthocyanins are produced in vegetative organs. Yet, the appearance of red coloration during autumn leaf senescence, due to anthocyanin accumulation, is the most striking and widely appreciated of all of these phenomena. These autumn displays vary in intensity in different parts of the world, but are widespread in temperate deciduous forests. The spectacular shows of autumn color in forests of northeastern North America are major sources of revenue from tourism. Indeed, many readers will be attracted to this book because of past experiences of collecting colored autumn leaves during childhood, and living in settings where the forests change in color every year. Despite the high concentration of universities and other academic centers near such forests, surprisingly little research has been conducted on the phenomenon, and our base of scientific knowledge is rather meager. In this chapter I review this body of research, emphasizing recently published work (and some in which I have been involved), and will suggest directions for future research.

A. IMPORTANCE

Red and yellow foliage is produced during leaf senescence by different species in all temperate deciduous forests. No comparison of the extent and systematic distribution of color production has been completed, yet it is evident that the degree of color production is highly variable. The production of autumn coloration is perhaps most dramatic in the mixed deciduous forests of northeastern United States, particularly in New England. There, the red foliage is so pronounced that it is easily detectable by satellite remote sensing (Boyer *et al.*, 1988). However, the show of autumn coloration in the forests of the Great Smoky Mountains may be equally spectacular (Palevitz, 2001). Those in Europe and temperate Asia may be less pronounced, at least for the presence of brilliantly red-senescent trees and shrubs.

The autumn display of vermillion is an economic engine, drawing millions of visitors to these forests during the autumn, worth hundreds of millions of dollars in tourist revenues (Hendry, 1988). As a consequence, predictions of the timing and intensity of color production are of economic value. Local residents predict the intensity of color production based on the frequency of rainfall in the late summer months, as well as the frequency of cold and sunny days early in the autumn (John O'Keefe, personal communication).

In virtually all cases, the production of red foliage is due to the accumulation of anthocyanins in leaf tissues. The yellow colors are due to the persistence of xanthophyll pigments, made noticeable through the degradation of chlorophyll.

B. HISTORY

Naturalists and scientists have commented on autumn coloration for centuries, some of this documented by Wheldale's (1916) early review, along with the poetic descriptions of Kerner von Marilaum (1897). Early research on red coloration in New England forests was completed by Smith (1901), and Gertz (1906) included microscopic observations of senescent leaves in his extensive European survey of anthocyanin distribution in vegetative organs. Observations of this color production helped feed the speculations of late 19th century scientists arguing about the various possible functions of anthocyanins (see Lee and Gould in this volume). The small body of research published during the past 50 years will be discussed under specific headings in this chapter.

C. ANTHOCYANINS AND OTHER PIGMENTS

As a rule, orange-red coloration during senescence is caused by the synthesis of anthocyanins. There are a few cases of red coloration caused by xanthophylls (Ida *et al.*, 1995), but these are rare indeed. Additional research may reveal the production of even more unusual pigments during leaf senescence (see Lee in this volume), but this is also likely to be extremely unusual. Betacyanins are responsible for red coloration in leaves of species within the Caryophyllales, but this is apparently restricted to the formative stages in leaf ontogeny, or to foliage under physiological stress (Lee and Collins, 2001). Moreover, the woody species of Caryophyllales are almost entirely tropical evergreens. Given the higher metabolic cost of the nitrogenous betacyanins, their production during senescence should be less likely than anthocyanins.

The diversity of anthocyanin pigments in flowers is quite high; more than 250 different structures have been identified (Harborne and Grayer,

1988). Anthocyanin molecules vary in the structure of the pigment aglycone (anthocyanidin), as well as in the types of sugars and their positions of attachment on the anthocyanidin. In contrast, the structures of anthocyanins in senescing leaves are remarkably conserved; almost all are cyanidin based. Early work reported anthocyanins as predominantly cyanidin-3-glucosides (Ishakura, 1972). Given the application of the more sensitive and rapid contemporary techniques of high pressure liquid chromatography (HPLC), it is likely that other sugar moieties will be found in these leaf pigments with time (Ji *et al.*, 1992). Still, the low diversity of types of anthocyanins in leaves, compared to flowers, is striking.

D. UNMASKED OR SYNTHESIZED?

There is a common misperception about the origin of anthocyanins during leaf senescence. Textbooks frequently state that anthocyanins are permanently present during the leaf life span, their color production being unmasked by the breakdown of chlorophylls during senescence (Moore *et al.*, 1998; Uno *et al.*, 2001). The alleged accumulation of these pigments (Matile, 2000) is consistent with the classical argument of the accumulation in vacuoles of those by-products of metabolism with no physiological function other than carbohydrate overflow (Fraenkel, 1959; Luckner, 1984).

The unmasking hypothesis is scientifically unsound, however. Sanger (1971) had shown that anthocyanins are synthesized *de novo* in leaves during senescence. Lee *et al.* (2002) found no anatomical evidence of anthocyanins in mature leaves that accumulated these pigments in senescence (the 18 species studied in detail and referred to in other parts of this chapter are listed in Table I). Moreover, color production by anthocyanins in the cell vacuoles of mature leaves would modify the entire leaf color, as it does for certain tree cultivars, as the copper beech (*Fagus sylvatica* var. *atropunicea*) or purple varieties of the Japanese maple (*Acer palmatum* var. *atropurpureum*). So, these pigments would be visible in mature leaves. Extensive research in photobiology and in molecular developmental genetics (see Lee and Gould this volume), where anthocyanin induction and synthesis is a model system of research, has shown that anthocyanin synthesis is inducible in mature leaves by a variety of environmental and anthropogenic factors, (Chalker-Scott, 1999; Chalker-Scott in this volume). Clearly, anthocyanin synthesis during the senescence of red-foliage plants contrasts strongly with the production of coloration in yellow-foliage plants, which result from the unmasking of xanthophyll pigments during chlorophyll degradation.

TABLE 1

Leaf senescence at the Harvard Forest, in Central Massachusetts USA (Lee et al., 2002). Eighteen species (with families) are listed, the species analysed for pigmentation and mentioned in the text; 16 species were monitored for phenological changes at weekly intervals 1991–1998 (except for 1992)

Species	Family	Mean time (\pm SD) to 50% leaf fall (Julian day) ^a	Anthocyanin distribution ^b					
			EP	PP	SM	UE	TS	
Anthocyanic								
<i>Acer rubrum</i> L.	Aceraceae	280 \pm 3	1	4	1	1	0	
<i>Acer saccharum</i> H. Marsh	Aceraceae	293 \pm 2	0	3	0	0	0	
<i>Cornus alternifolia</i> L. Fil.	Comaceae	287 \pm 3	0	3	0	0	0	
<i>Fraxinus americana</i> L.	Oleaceae	285 \pm 4	0	4	0	0	0	
<i>Prunus serotina</i> J. F. Ehrh.	Rosaceae	290 \pm 3	1	0	0	0	0	
<i>Quercus rubra</i> L.	Fagaceae	299 \pm 4	0	3	1	0	0	
<i>Vaccinium corymbosum</i> L.	Ericaceae	296 \pm 4	0	4	0	0	0	
<i>Viburnum alnifolium</i> H. Marsh	Caprifoliaceae	288 \pm 6	0	4	0	0	0	
<i>Viburnum cassinoides</i> L.	Caprifoliaceae	298 \pm 7	0	4	1	0	0	
Non-anthocyanic								
<i>Acer pensylvanicum</i> L.	Aceraceae	288 \pm 5						
<i>Betula alleghaniensis</i> Britton	Betulaceae	281 \pm 4						
<i>Betula populifolia</i> H. Marsh	Betulaceae	284 \pm 4						
<i>Castanea dentata</i> (H. Marsh)	Fagaceae	293 \pm 4						
Borkh.								
<i>Fagus grandifolia</i> J. F. Ehrh.	Fagaceae	306 \pm 3						
<i>Hamamelis virginiana</i> L.	Hamamelidaceae	287 \pm 4						
<i>Ilex verticillata</i> (L.) A. Gray	Aquifoliaceae	304 \pm 4						
<i>Populus grandidentata</i> Michx.	Salicaceae	—						
<i>Populus tremuloides</i> Michx.	Salicaceae	—						
Overall mean		290 \pm 6						

^a Leaf drop is given as time in Julian days (consecutive days from the first of the year) to achieve 50% leaf fall. Figures are a mean of three lagged trees for each species.

^b Scale of intensity of 1–4, where 1 = a few cells lightly pigmented and 4 = all cells densely pigmented. EP = adaxial epidermis; PP = palisade parenchyma; SM = spongy mesophyll; UE = abaxial epidermis; TS = trichomes/scales.

II. WHAT INFLUENCES PIGMENT DISTRIBUTION

Anthocyanin synthesis during leaf senescence is highly influenced by environmental factors. Concentrations can vary across different parts of a leaf, particularly in certain species such as *Acer rubrum* in eastern forests of the United States. In all species the concentration of anthocyanins varies among leaves from different parts of the tree or shrub crown, and is reduced by shading (Wheldale, 1916; Lee *et al.*, 2002). The shading of one part of a leaf by another suppresses anthocyanin production. Lee *et al.* (2002) also showed that alteration in red:far-red wavelengths (that could change phytochrome equilibria) had no perceptible effect on anthocyanin production.

Individual trees vary in production of anthocyanins during senescence. Such differences could be due to physical effects at microsites and/or genetic variation. For instance, some populations of aspen (*Populus tremuloides*) produce red leaves (Chang *et al.*, 1989) while most turn yellow during senescence.

Anthocyanin synthesis during the autumn is associated with leaf senescence and chlorophyll degradation. Both the overall process of leaf senescence (Smart, 1994; Quirino *et al.*, 2000) and chlorophyll degradation (Matile *et al.*, 1999; Thomas *et al.*, 2001) are highly coordinated and regulated processes. Given the association of anthocyanin synthesis with leaf senescence, chlorophyll concentration is a good physiological marker of production among different species (Wolf, 1956; Sanger, 1971; Koike, 1990; Collier and Thibodeau, 1995). The initial measurements of chlorophyll contents of 18 species at Harvard Forest (species listed in Table I), late in the growing season, indicate that considerable chlorophyll had already been lost by some of the species before anthocyanins were synthesised. Generally, anthocyanins began to appear in the leaves with about half of the initial chlorophyll ($\sim 20 \mu\text{g cm}^{-2}$) remaining, but there is considerable variation among leaves, individuals and species.

A. PHENOLOGY

The timing of anthocyanin production during senescence varies among different species, as documented among nine species by Lee *et al.* (2002; Table I). Some species began senescence earlier than others, the earliest being *Acer rubrum* and the latest being *Fagus grandifolia* (whose leaves tend to be retained by the tree). What is most interesting in this phenology, from weekly observations by John O'Keefe over a 10-year period, is the strong consistency in the timing and order of senescence among these species from year to year. The intensity of color production, which is

a combination of the number of leaves retained on the tree at peak coloration as well as the intensity of anthocyanin synthesis for the red leaves, varied from year to year. There is much speculation by residents living in the region as to what factors control the intensity of color. Adequate rainfall (but not too much) late in the growing season appears to be important, as do clear sunny days and cool nights in the period immediately preceding the induction of anthocyanin synthesis (Kozlowski and Pallardy, 1997).

B. SYSTEMATIC VARIATION

Some species with senescing red foliage will be found in virtually any forest. A small percentage of red-senescing trees have been documented in both tropical evergreen and deciduous forests (Lee and Collins, 2001; Lee in this volume). In forests of northeastern North America, this percentage may be much higher. For instance, at the Harvard Forest in central Massachusetts 62 of 89 woody species accumulated anthocyanins during senescence (Lee *et al.*, 2002; Plate 6). In some cases these accumulations resulted in spectacular color displays, but in others the combination of anthocyanins and residual chlorophyll produced brown coloration that appeared much like dead tissue. Some evergreen species may accumulate anthocyanins during the fall and winter and lose them at the beginning of the next growing season (see Starr and Oberbauer in this volume for such changes in Arctic evergreen vegetation, and Gould *et al.* this volume for New Zealand temperate evergreen forests).

The leaves of the large majority of these species produced anthocyanins in the mesophyll, predominantly in the palisade layer. For instance, in *Quercus rubra*, anthocyanins accumulated primarily in the palisade, late in the chlorophyll breakdown process (Plate 7). Anthocyanins were easy to detect in the vacuoles of these cells. Certain families may be particularly important sources of species producing red autumnal coloration, certainly the Aceraceae, Nyssaceae, Caprifoliaceae and Ericaceae. Others, such as the Betulaceae, or even sub-families, as the Fagoideae (but not the Quercoideae) are notable for the production of yellow autumn foliage and an absence of anthocyanins. In tropical forests certain families are particularly conspicuous for red coloration during senescence: particularly the Combretaceae and Lythraceae (see Lee in this volume). The brilliant senescent red leaves of the Indian almond (*Terminalia catappa*) are conspicuous in landscapes throughout the tropics, and in coastal forests of Southeast Asia. Thus the systematic composition of such forests may influence the patterns of color production.

C. CLIMATE

Classical authors noted the correlation between red foliage and temperature. Observations of the high incidence of red colors in alpine vegetation in Europe fed the arguments about anthocyanin function in leaves during the latter part of the 19th century (Wheldale, 1916; Kerner von Marilaum, 1897). Although less-studied, both the deciduous and the evergreen species of Arctic tundra plant communities turn red in the autumn (Starr and Oberbauer in this volume). Experimental evidence for the correlation between pigmentation and latitude (which would be equivalent to drop in temperature) was obtained by Townsend (1977) who grew accessions of the red maple (*Acer rubrum*) from different latitudes in a common garden. He observed that those from higher latitudes produced more autumn coloration. Hoch *et al.* (2001) concluded from a literature survey that among genera of woody species from North America and Europe, those species in northern North America were the more likely to produce anthocyanins during senescence. They also concluded that the milder maritime climates of European forests did not select for pigment production. However, in a survey of leaf anthocyanins in *Acer* reported by Ji *et al.* (1992) 67 out of 91 species (out of a total for the genus of approximately 200) produced pigments during senescence. Anthocyanic species were found at the north and south extremes of the geographical range, even in transient coloration among evergreen species from Southeast Asia.

Climatological gradients may result in ecotypic differentiation, and ultimately the evolution of species with these pigmentation differences. Perhaps some of the differences in autumn coloration of temperate deciduous forests may be due to phylogenetic constraints within taxa that are geographically distributed among these forests (Lee in this volume).

D. ECOLOGICAL CORRELATES

Red foliage occurs in all of the plant functional groups that make up temperate deciduous forests. These include trees, shrubs, and perennials, all of these of varying successional stages. However, red leaf color may be less common among annuals in such forests (personal observation). At the Harvard Forest, the majority of species produced anthocyanins during senescence, including species among all of the functional groups listed above (Lee *et al.*, 2002).

In their review of the physiological significance of anthocyanins during autumnal leaf senescence Hoch *et al.* (2001) argued that species acclimated to higher insolation, such as early successional species, should be less susceptible to photoinhibition and damage, and therefore

less likely to produce anthocyanins as additional protection during autumn leaf senescence. There is considerable evidence that plants adapted to high insolation are less susceptible to photoinhibition, and employ other mechanisms, such as the xanthophyll cycle pigments, to protect against photodamage (reviewed by Hoch *et al.*, 2001), also well-documented in high-altitude species (Streb *et al.*, 1998). Koike (1990) found that 16 early successional tree species in temperate deciduous forests in Japan began leaf senescence from the inside of their crowns towards the outside, and did not produce anthocyanins. Conversely, 14 late successional species began leaf senescence from the outside of their crowns and developed anthocyanins.

In their comparison of 18 species at Harvard Forest, Lee *et al.* (2002; Table I) did not observe a relationship between successional status and anthocyanin production. Also, some of the more shade tolerant species (theoretically the more susceptible to photoinhibition in high sunlight), such as *Acer pensylvanicum* and *Hamamelis virginiana*, did not produce anthocyanins during senescence. Another early successional species, *Prunus serotina*, was very weakly anthocyanic during senescence, compared to other species. However, it is important to remember that the New England forests, most famous for their spectacular autumn coloration, are not mature forests, since most of the region was converted to farmland in the 18th and 19th centuries.

At Harvard Forest, the nine red-senescing (and anthocyanic) species differed from the nine yellow-senescing species in several ways. Senescing leaves of the anthocyanic species had:

1. lower chlorophyll a/b ratios; and
2. greater mass/area.

Such differences between the two pigmentation patterns cannot be explained in terms of the successional status of the species.

III. THE FUNCTION OF ANTHOCYANINS DURING LEAF SENESCENCE

The chapters in this volume review past hypotheses on the possible physiological and other adaptive functions of anthocyanins in leaves. Authors have focused on five potential mechanisms that are objects of current research. These are:

1. photoprotection against photoinhibition and photodamage;
2. protection against UV-damage;
3. anti-oxidation;
4. osmotic regulation; and
5. biological interactions.

All of these potential explanations must confront a particular challenge in the case of autumn leaves: protection by anthocyanins appears an expensive option for leaves at the end of their life span, when photosynthetic capacity is low. Although anthocyanins have high extinction coefficients and can alter leaf optical properties at low concentrations (and are also extremely effective antioxidant molecules), they are the terminus of an elaborate biosynthetic pathway and their metabolic costs are relatively high. Thus, it seems likely that the benefit(s) to plants would extend beyond a few weeks at the end of the growing season.

A. PHYSIOLOGICAL EXPLANATIONS

The highly coordinated processes of leaf senescence (Smart, 1994; Quirino *et al.*, 2000) and chlorophyll degradation (Matile *et al.*, 1999; Thomas, 2001) appear to increase the resorption of nutrients, particularly nitrogen, back into woody plant tissues. Since almost all of leaf nitrogen is associated with photosynthesis, and much of that with chlorophyll in membrane-bound complexes, chlorophyll degradation is particularly important. Loss of nutrients from leaf fall varies among different plants and ecosystems (Aerts, 1996; Killingbeck, 1996). Recovery can be assessed in terms of its efficiency (or resorption efficiency of Killingbeck) but only if there is an accurate assessment of the maximum nitrogen (N) content during the leaf life span. Since the processes of senescence may begin during the growing season, multiple measurements may be necessary to determine this maximum. Then the efficiency can be assessed as:

$$\frac{(\text{N in leaves at maturity} - \text{N in falling leaves})}{\text{N in leaves at maturity}}$$

Resorption efficiencies averaged 50.3% across a large sample of species (Aerts, 1996). Because of the difficulty determining maximum N concentrations, Killingbeck (1996) has advocated the use of the term proficiency, which is the nitrogen content of the falling leaves. Values of N resorption proficiency in deciduous woody species varied from 0.46 to 1.90%.

Hoch *et al.* (2001), in an extensive survey of the literature, argued that anthocyanin accumulation during leaf senescence could increase N proficiency of deciduous plants by one or more protective mechanisms. A similar argument was raised independently (Feild *et al.*, 2001) in an experimental study of photoprotection during leaf senescence. Anthocyanins, through photoprotection by absorbing in the blue-green wavelengths could reduce photoinhibition and photodamage in senescing leaves (Smillie and Hetherington, 1999). The antioxidant properties of

anthocyanins could also scavenge reactive oxygen species (ROS) produced by a damaged photosynthetic apparatus (see Gould *et al.* this volume). This direct protection would facilitate a more efficient removal of nitrogen from mesophyll cells (where anthocyanins accumulate) back into woody stems. Movement of nitrogen requires adequate storage of carbohydrates for energy production and a permissible osmotic environment. Thus, although continued photosynthesis may not be critical for the overall carbon balance of the plant, it may provide a source of energy to allow the export of nitrogenous compounds to occur (Collier and Thibodeau, 1995; Solecka *et al.*, 1999; Merzlyak and Chivkunova, 2000). Indirect evidence supporting this hypothesis comes from a variety of sources, reviewed by Hoch *et al.* (2001). The hypothesis predicts a correlation of anthocyanin synthesis with chlorophyll decline. It also predicts that additional protection would be important where the potential of photodamage is increased. The high irradiances and low temperatures of autumn days, with high photosystem activity and the curtailing of dark CO₂ fixation by low temperatures, would increase potential for damage (Huner *et al.*, 1998).

Lee *et al.* (2002) tested for persistence in photoprotection of anthocyanic leaves in trees of nine red and nine yellow-senescing trees (Fig. 1). Using chlorophyll concentration as an indicator of senescence in leaves sampled during the autumn, they found a log-linear relationship in reduction of Fv/Fm with chlorophyll reduction. However, the

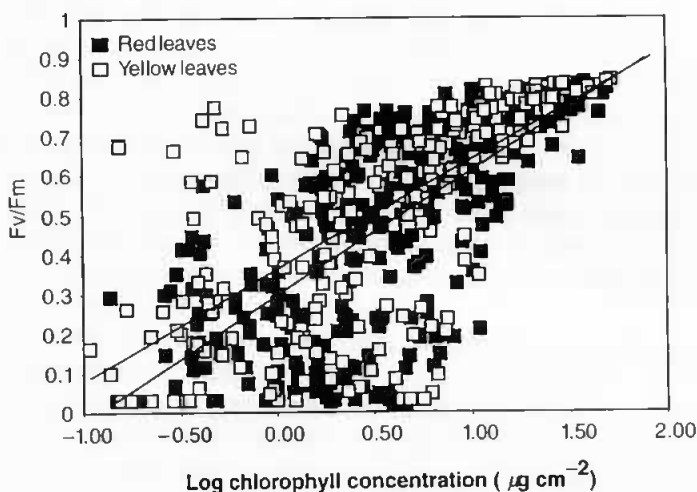


Fig. 1. Relationship between log of chlorophyll content ($\mu\text{g cm}^{-2}$) and Fv/Fm from leaves collected before dawn of nine red-senescing and nine yellow-senescing species from Harvard Forest, as listed in Table 1 (Lee *et al.*, 2002). A lower value for Fv/Fm indicates a reduction in quantum efficiency, most likely the consequence of photoinhibition.

slope of reduction of the red-senescing species was not significantly different from that for the yellow senescing species. Since the leaves used for these measurements were collected just before dawn, the plants had had ample opportunity for recovery from photoinhibition due to exposure the previous day. Feild *et al.* (2001, and Timmins *et al.* in this volume) conducted a more direct experiment by estimating photoinhibition and recovery in senescing red and green leaves of red-osier dogwood (*Cornus stolonifera*). They observed a significant reduction in photoinhibition, and more rapid recovery, in red leaves. These results give direct evidence for protection against photoinhibition in these senescing deciduous leaves. Krol *et al.* (1995) had seen a similar protective function in evergreen needles of *Pinus banksiana* seedlings, and Post and Vesik (1992) had detected similar photoprotection in Antarctic bryophytes. Starr and Oberbauer (in this volume) give supporting evidence for the operation of this protective mechanism in evergreen tundra plants. Other physiological mechanisms may also be important (see Chalker-Scott this volume in particular), but there is no direct supporting evidence at present.

A critical test for the operation of one or more of these protective mechanisms for the long-term survival of red-senescing plants is obviously to look at patterns in nitrogen resorption. Protection by anthocyanins should result in reduced nitrogen contents of falling leaves, and therefore more resorption into parent tissues. Obtaining such evidence is complicated by the effect of shading on anthocyanin production and, at the same time, reduced risk of photoinhibition. Using leaves that vary in anthocyanin production within a crown may not reveal any differences in nutrient resorption; Feild *et al.* (2001) failed to detect any differences in nitrogen resorption in senescent leaves of *C. stolonifera*.

Using a broader comparative approach, Lee *et al.* (2002) measured nitrogen in falling leaves of individuals of nine red- and nine yellow-senescing woody species at Harvard Forest (Table I for species compared). The nitrogen contents of senescent red-leaved species overall were reduced relative to those of the yellow, but the difference was not statistically significant ($P = 0.084$ from pairwise comparison by *t* test). However, when leaf samples of individuals were compared (Table II), leaf nitrogen (as a percentage of dry tissue mass) in senescent leaves was significantly and negatively correlated with anthocyanin concentration. In these comparisons, anthocyanins in senescent leaves were also correlated with mass/area of senescent leaves, as well as negatively correlated with nitrogen content in mature leaves of the same plants. However, anthocyanin content was not correlated with nitrogen content in leaves per unit area. Obviously, nutrient predictions of the protection hypothesis need more extensive testing.

TABLE II

Pearson product correlations of leaf anthocyanin contents and other leaf characters in pooled leaf samples of three individuals from each of the 18 species at Harvard Forest, listed in Table I (Lee et al., 2002). All measurements were from senescent leaves, except for % nitrogen in mature leaf mass (%NM)

	Leaf characters ^a					
	ANT	CAS	MAS	%NS	%NM	NAS
ANT	-	0.004 ^f	0.362	-0.334	-0.361	-0.052
Significance ^b		ns	**	*	**	ns
CAS		-	0.404	0.083	0.308	0.353
Significance			**	ns	**	*
MAS			-	0.067	0.105	0.741
Significance				ns	ns	**
%NS				-	0.598	0.691
Significance					**	**
%NM					-	0.495
Significance						**
NAS						-

^a ANT = Anthocyanin concentration ($\mu\text{g cm}^{-2}$); CAS = chlorophyll/area ($\mu\text{g cm}^{-1}$); MAS = mass/area (mg cm^{-2}); %NS nitrogen as a % of senescent (dry) leaf mass; %NM = %nitrogen in mature leaf mass; NAS = nitrogen/area ($\mu\text{g cm}^{-2}$)

^b Values in bold are significant, with * = < 0.05, ** = < 0.005 and ns = not significant.

B. ECOLOGICAL EXPLANATIONS

Because of their strong visual signal (at least for animals with some color vision) it is natural to think of anthocyanic coloration as having some ecological significance. There has been some research and speculation on the ecological significance of anthocyanins of developing leaves in reducing herbivory (see review by Lee and Gould this volume), but avoiding herbivory on leaves that are about to abscise makes little sense. Nevertheless, there are two hypotheses invoking ecological interactions which would benefit the plant in subsequent growing seasons.

1. The Fruit Flag Hypothesis

Stiles (1982) proposed that brilliant leaf color in the autumn could attract birds to ripe fruits associated with the color, promoting dispersal of propagules and enhancing the colonization success of the species. Birds are particularly attracted to red colors for flower visitation and pollination, as well as frugivory and seed dispersal. Such a mechanism requires that fruits be present on the plant at the end of the growing season when the leaves become visible. Obvious candidates for such a mechanism of attraction and dispersal are the sumacs (*Rhus* spp.) with their long infructescences at the ends of branches, associated with the subtending and brilliantly colored leaves. Several such species of *Rhus* were

observed at Harvard Forest, all with high concentrations of anthocyanins in the palisade layer of their leaves. The most brilliantly colored autumn species there was a shrub, *Euonymus atropurpureus*, an invasive exotic common in the understory at forest fringes. In this species anthocyanin is produced at high concentrations in the adaxial epidermis predominantly when chlorophyll has been removed from the leaf mesophyll, producing the intense red color. The fruit flag hypothesis is certainly viable for plants that produce animal-dispersed propagules at the end of the growing season, but it can only account for pigment production in a small minority of trees in temperate deciduous forests. At Harvard Forest, most species produce fruits that ripen before autumn senescence begins, or their fruits are dispersed by wind or other mechanisms.

2. *The Defensive Signal Hypothesis*

If red leaf coloration were associated with lack of palatability of leaves or an increase in defensive compounds, such color could warn away potential herbivores. Coley and Barone (1996) summarized research that demonstrated such a defensive role in the flushing leaves of tropical rainforest plants. For those species, the putative warning signals had the obvious advantage of protecting expanding leaves at the beginning of their life spans. However, such a warning would not make sense for the protection of dying leaves. Hamilton and Brown (2001) adapted this hypothesis to the protection of deciduous-temperate tree species against the egg-laying and later herbivorous activity of aphids. They hypothesized that bright autumn coloration, associated with an increase in defensive chemistry, among different species and between populations or individuals within a single species, could deter late season visits by aphids. Such insects oviposit in the autumn, and the eggs hatch the following spring and increase herbivory pressure in the following year. Their test of this hypothesis was an analysis of published research, primarily a list of 262 tree species obtained from field guides which described the types and degrees of autumn coloration, and an exhaustive global compendium of aphids attacking trees (Blackman and Eastop, 1994). In their statistical regressions of autumn color against aphid diversity, they were able to account for potential phylogenetic influence (see Lee this volume). A model for these types of interactions was published by Archetti (2000) and was shown to be plausible.

Hamilton and Brown (2001) found a strong correlation between the degree of yellowness and herbivory by aphids (either all aphids or single specialist species), and a weaker relationship between redness and single specialist species. When they looked for regressions against autumn leaf color and attack by specialist aphids, and controlled for other variables, they found stronger regressions, but more so for yellow than for red

color. They concluded that autumn color functions as a signal of defensive commitment against autumn colonization and future herbivory by aphids. In support of this hypothesis, there is good evidence that aphids discriminate among individuals within tree species, as shown by Furuta (1990) for *Acer palmatum*.

The defensive signal hypothesis for anthocyanins in senescing leaves is deficient in several areas. First, the strongest signal against egg-laying is bright yellow coloration, the region of the spectrum where most aphids have sensitive color vision (Hamilton and Brown, 2001). Yellow color is the result of the differential rates of breakdown of chlorophylls and xanthophylls in chloroplasts. The rate of xanthophyll loss is the same in red- and yellow-senescing species at the Harvard Forest (Lee *et al.*, 2002; Fig. 2). Retention of xanthophylls in detached leaves does represent a carbon loss to these trees, but it is metabolically less expensive than the nitrogenous chlorophylls. Given the strong signal provided by yellow leaves, it seems illogical that anthocyanins would confer an additional advantage, unless certain specialist aphids have evolved color sensitivity in the red end of the spectrum. Finally, from a plant's standpoint, there is no evidence of a relationship between autumn color and defensive chemistry in leaves of any shrub or tree, let alone between autumn color and defensive compounds (such as tannins) in the following year.

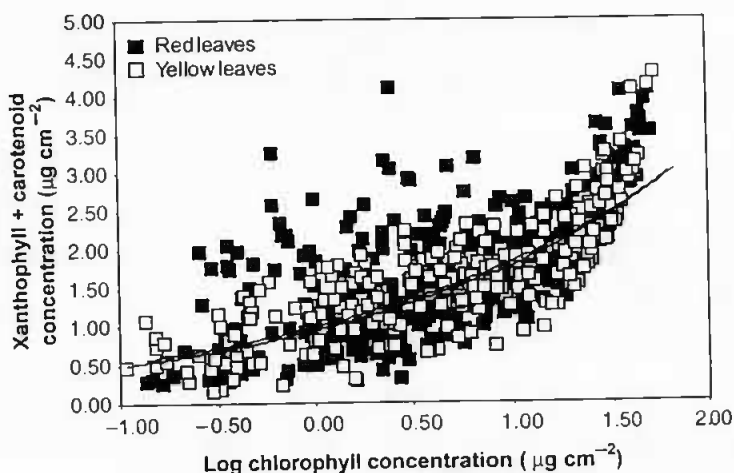


Fig. 2. Relationship between log of chlorophyll content ($\mu\text{g cm}^{-2}$) and total xanthophyll concentration ($\mu\text{g cm}^{-2}$) for leaves collected from the nine red-senescing and nine yellow-senescing species from Harvard Forest, as listed in Table I (Lee *et al.*, 2002). These plots indicate that the two species groups lose xanthophylls during senescence in an identical manner.

IV. FUTURE RESEARCH

We now have some good working hypotheses on how anthocyanins might function during leaf senescence, and these make predictions that can be tested by manipulating plants and measuring them in natural settings. We still need to learn more about physical factors that promote the production of anthocyanins, as well as the patterns (and their persistence) in anthocyanin production in populations of trees in forests. For instance, we need more empirical data on the intensity of anthocyanin coloration during autumn, and its relationship to climate and weather patterns during the year.

We need a plant that can be studied as a research system. The plant must grow quickly, be propagated easily, and be amenable to growth under greenhouse/growth chamber conditions. For such a plant we need to select and characterize mutants for deficiencies in anthocyanin production, so that we can compare their physiology under different stresses, such as cold and sunny conditions. Such a system would help us distinguish among the various physiological explanations that have been proposed for anthocyanin function. Ultimately, such a plant could be genetically engineered not to produce anthocyanins, then compared with normal wild type plants.

We need to study the nutrient dynamics, particularly in nitrogen flux, for species in forests with autumn coloration, to examine more critically the possibility of reduced nitrogen retention among species that produce anthocyanins during senescence. Again, comparisons among individuals within a species that vary in the production of anthocyanins would be a better test of the possible protective role of anthocyanins. Comparative approaches, where many individuals among numerous species with varying color production during senescence, would be a useful counterpoint to the more reductionist approaches.

We also need to pursue questions raised by the research on biological interactions. We need to examine carefully the color vision of aphid species that seem to specifically feed on anthocyanic trees. We also need to assess the relationship between anthocyanin production and secondary compound production (such as tannins) in natural populations and among different tree species.

It would also be interesting to look at the production of anthocyanins during leaf senescence in annual plants. Is such color production common? If so, what advantage would it be for a plant about to perish? Could such coloration contribute to higher nitrogen content (and survival) in seeds?

Finally, if anthocyanins are found to play a definitive role in the protection of plants during autumn senescence, then what alternative adaptive strategies have been selected by plants that do not turn red during leaf senescence?

V. CONCLUSION

Although the history of interest in autumn coloration stretches back over the centuries, only recently has that interest resulted in serious scientific study of the phenomenon. We now have some working hypotheses about anthocyanin function and clear indications on where to turn our attention next. The future looks bright for a much better understanding of this phenomenon in the near future.

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