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Pigment dynamics and autumn leaf senescence in a New England deciduous forest, eastern USA

David W. Lee,[1,2]* John O'Keefe,[3] N. Michele Holbrook[4] and Taylor S. Feild[5]

[1]*Department of Biological Sciences, Florida International University, Miami, Florida 33199, USA,* [2]*Fairchild Tropical Garden, Miami, Florida 33156, USA,* [3]*Harvard Forest, Petersham, Massachusetts 01366, USA,* [4]*Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts 02138, USA and* [5]*Department of Botany, University of Toronto, Toronto M5S 3B2, Canada*

The leaves of woody plants at Harvard Forest in Central Massachusetts, USA, changed color during senescence; 70% (62/89) of the woody species examined anatomically contained anthocyanins during senescence. Anthocyanins were not present in summer green leaves, and appeared primarily in the vacuoles of palisade parenchyma cells. Yellow coloration was a result of the unmasking of xanthophyll pigments in senescing chloroplasts. In nine red-senescing species, anthocyanins were not detectable in mature leaves, and were synthesized de novo in senescence, with less than 20 μg cm^{-2} of chlorophyll remaining. Xanthophyll concentrations declined in relation to chlorophyll to the same extent in both yellow- and red-leaved taxa. Declines in the maximum photosystem II quantum yield of leaves collected prior to dawn were only slightly less in the red-senescing species, indicating no long-term protective activity. Red-leaved species had significantly greater mass/area and lower chlorophyll *a*/*b* ratios during senescence. Nitrogen tissue concentrations in mature and senescent leaves negatively correlated to anthocyanin concentrations in senescent leaves, weak evidence for more efficient nitrogen resorption in anthocyanic species. Shading retarded both chlorophyll loss and anthocyanin production in *Cornus alternifolia*, *Acer rubrum*, *Acer saccharum*, *Quercus rubra* and *Viburnum alnifolium*. It promoted chlorophyll loss in yellow-senescing *Fagus grandifolia*. A reduced red : far-red ratio did not affect this process. Anthocyanins did not increase leaf temperatures in *Q. rubra* and *Vaccinium corymbosum* on cold and sunny days. The timing of leaf-fall was remarkably constant from year to year, and the order of senescence of individual species was consistent.

Key words: anthocyanin; function; leaf senescence; temperate deciduous forest.

INTRODUCTION

The autumn coloration of temperate deciduous forests, particularly in the eastern USA, is a spectacular and yet poorly studied phenomenon. Red coloration in autumn foliage is primarily a result of the production of the anthocyanin cyanidin-3-glycoside (Ishikura 1972). Anthocyanin synthesis in vegetative organs is induced by different environmental factors (Mol *et al*. 1996; Chalker-Scott 1999), and anthocyanins increase in concentration during senescence (Sanger 1971; Chang *et al*. 1989).

Anthocyanin production and function in senescent leaves has been discussed often but researched little (Gould & Lee 2002). Their functions in photoprotection and in increasing leaf temperatures was first suggested in the nineteenth century (Wheldale 1916). Smith (1909) hypothesized that anthocyanins elevated leaf temperature and increased rates of metabolism, but more recent tests have not shown such changes (Lee *et al*. 1987; Gould *et al*. 1995). By the mid twentieth century, the photoprotection hypothesis was largely forgotten, and anthocyanin synthesis was considered to be the result of carbohydrate 'overflow' during the recycling of secondary compounds, or one of the by-products

*Author to whom correspondence should be addressed. Email: leed@fiu.edu

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moved to vacuoles during senescence (Ford 1984; Luckner 1984).

There is now growing interest in the potential for photoprotection by anthocyanins, although their role in ultraviolet protection has been largely discounted (Lee *et al*. 1987). Gould *et al*. (1995) hypothesized that anthocyanins in the abaxial leaf surfaces of rainforest understory plants help protect the chlorophyll *b*-rich light harvesting complex II (LHC-II) of spongy mesophyll chloroplasts from photodamage by excess irradiance. Post (1990) and Post and Vesk (1992) demonstrated the protective effect of an anthocyanin-like pigment in Antarctic bryophytes. Krol *et al*. (1995) showed that anthocyanins in the needles of *Pinus banksiana* increased tolerance to photo-inhibition at high irradiance and low temperatures; and Sharma and Banerji (1981) measured enhanced Hill activity in leaves of two species with accumulations of anthocyanin. In addition to photoprotection, anthocyanins are strong free-radical scavengers (Yamasaki *et al*. 1996; Yamasaki 1997), and could prevent photo-oxidative damage in sensitive tissues (Gould *et al*. 2002). Cyanidin-glucosides, most prevalent in leaf senescence, are particularly potent anti-oxidants (Tsuda *et al*. 1994).

Photoprotection in senescing leaves by anthocyanins should be unimportant because of the minimal future carbon gain in leaves about to fall (Koike 1990). Furthermore, anthocyanins are quickly shunted to the vacuoles by way of a glutathione pump (Alfenito *et al*. 1998) and would not concentrate near chloroplasts in the cytoplasm. However, photoprotection could aid in the resorption of nutrients, particularly nitrogen and phosphorus, by reducing the oxidative activity of the breakdown products of photosynthesis sequestered in vacuoles during the orderly breakdown of chlorophyll (Matile *et al*. 1999). Free radicals could disrupt the movement of nitrogen and/or phosphorus from leaves back into branches. Thus, anthocyanins, through photoprotection and free-radical scavenging (Hoch *et al*. 2001), could increase the efficiency of nutrient resorption and reduce the residual nitrogen or phosphorus in fallen leaves (Aerts 1996; Killingbeck 1996). Feild *et al*. (2001) have shown that anthocyanins in senescing leaves of *Cornus stolonifera* influence chlorophyll fluorescence kinetics, suggesting a photoprotective function.

Other functions of anthocyanins are possible during autumn senescence: (i) responses to stress, particularly cold (Steponkus & Lanphear 1969; Huner *et al*. 1998); (ii) future herbivory (Archetti 2000; Hamilton & Brown 2001); and (iii) attraction of seed dispersers (Stiles 1982).

In the present study we report on the frequency of anthocyanic coloration in 89 woody species at Harvard Forest, central Massachusetts, USA. This data, for a single site, tests the predictions of certain hypotheses and provides empirical data at the community level for further research. We report on the changes in pigment concentrations in relation to physiology and nutrient status in leaves of nine yellow-senescing and nine red-senescing species. Finally, we monitored the timing of leaf senescence, the effects of shade on senescence in six species, and the effects of anthocyanins on leaf temperatures in two species. These comparative data lead to the following questions: (i) how common is anthocyanic coloration during senescence? (ii) at what stages are anthocyanins synthesized during senescence? (iii) are losses of xanthophyll pigments similar in the red- and yellow-senescing taxa? (iv) are increases in anthocyanin concentration during senescence associated with photoinhibition and/or photodamage, and with lower chlorophyll *a*/*b* ratios? (v) are increases in anthocyanin concentrations associated with higher leaf retention of nitrogen and/or phosphorus? (vi) do anthocyanins increase leaf temperature during senescence? (vii) are anthocyanin synthesis and chlorophyll loss influenced by reduced irradiance and reduced red : far-red wavelengths? and (viii) does the timing of senescence vary from year to year?

METHODS

Study site and focal species

The present study was conducted principally at the Harvard Forest, in the town of Petersham, MA, USA. Leaf samples of plants from forest properties and other locations within 30 km (Appendix I) were anatomically examined for the presence of anthocyanins during senescence. We studied woody species in most detail at the Prospect Hill Tract (42°32′N, 72°11′W, elevation 400 m above sea level) in the

transition-hardwoods–white-pine–hemlock forest type zone (Spurr 1956; Sipe & Bazzaz 1994). The soils at this site were low in nitrogen, and varied little at different locations (Compton & Boone 2000).

We examined leaves of 89 woody angiosperm species (three individuals each) for the presence of anthocyanins through microscopic observations (magnification × 200) of hand sections approximately 50 μm thick (Appendix I). This is 53% (89/169) of the total woody species diversity in all habitats at Harvard Forest (T. W. Sipe, unpubl. data, 1984); we examined the species found in sufficient number during the study period of August 1998–November 1998. Anthocyanins accumulate in the vacuoles of affected cells and are easily observed in very low concentrations (Lee & Collins 2001). Voucher specimens were deposited in the herbarium at Fairchild Tropical Garden, FL, USA.

Leaf and nutrient sampling

Pigment content, nutrient concentrations and variable fluorescence were measured in leaves of three plants of 18 species (Table 1) at the Prospect Hill Tract from the end of summer (7–10 September 1998) until the end of leaf-fall (1 November 1998). We tagged individuals randomly along access lanes in the forest, and collected leaves from lower branches exposed to some direct sunlight, except from individuals of *Acer pensylvanicum*, which were in dense shade. We collected five healthy leaves at the beginning of this period and five leaves at the end of senescence (by gently shaking leaves from the branches) for estimates of dry mass/area (dried in an oven at 80°C for 48 h), and a minimum of five leaves for tissue nutrient analysis. Leaf areas were estimated by tracing their outlines on paper and weighing the latter. Tissue carbon, nitrogen and phosphorus were determined in the pooled leaf samples. Total nitrogen and carbon were analyzed by dry combustion in a Carlo-Erba elemental analyzer (model NA 1500; Carlo-Erba Instruments, Milan, Italy; Bremner 1996; Nelson & Sommers 1996). Total phosphorus was analyzed colorimetrically after dry combustion (Environmental Protection Agency 365.1; Solorzano & Sharp 1980).

Pigment and fluorescence analysis

We analyzed each of (i) concentrations of chlorophyll *a* and *b*, (ii) total xanthophyll and anthocyanin, and (iii) photosynthetic efficiency (*Fv*/*F*m) in two leaves from each tree at the beginning of the study, six leaves from each tree during senescence, and two leaves from each tree at the time of leaf-fall (a total of 30 leaves for each species). All leaves were collected prior to dawn for measurement of dark-acclimated *Fv*/*F*m with an OS1-FL fluorometer (Opti-Sciences, Tyngsboro, MA, USA). Leaf disks were then cut from the same leaves with a cork borer for pigment extraction. Chlorophylls and total xanthophylls were extracted without tissue disruption in N,N-dimethyl formamide for 48 h at 3°C in darkness, using the equations for a 0.2-nm wavelength bandwidth (Wellburn 1994) and a Cary Model 219 spectrophotometer (Varian Inc., Palo Alto, CA, USA) set at that bandwidth, and calculating concentrations as μg cm^{-2}. Total chlorophyll and xanthophyll concentrations at leaf maturity were estimated from the two mature leaves of each individual, or $n = 6$. For senescence the same analyses were performed on the final two leaves collected, for $n = 6$. Because the difficulties in estimating chlorophyll *b* concentrations at very low concentrations make chlorophyll *a*/*b* ratios unreliable, we used six senescing leaves with chlorophyll concentrations greater than 1 μg cm^{-2} to estimate such ratios.

Anthocyanins were extracted with the same solvent acidified with 0.1 M HCI. We adapted this solvent rather then acidic methanol because of the need to extract without tissue disruption in the small areas of <1 cm^2 to compare with *Fv*/*F*m. We estimated total anthocyanins in μg cm^{-2} in leaves, subtracting for interference by phaeophytin (Murray & Hackett 1991; but using $0.16 \times A_{654}$, appropriate for this solvent). We modified the specific extinction coefficient for cyanidin-3-glucoside determined by Fuleki and Francis (1968) for this solvent at 525 nm by comparing the absorbance of extractions by acidic methanol of identical tissues: 3.8×10^4 l g^{-1} cm^{-1}. In rare cases of the most senescent leaves where extracts were brown, we also checked for additional interference of soluble tannins by bleaching extracts with 30% v/v H_2O_2 (Lee *et al*. 1987).

Table 1 Mass, pigment and nutrient concentrations, and photosynthetic efficiency in nine red-senescing (anthocyanic) and nine yellow-senescing woody species at Harvard Forest†

Species/Stage	Chlorophyll (μg cm^{-2})	(*a*/*b*)	Carotenoids (μg cm^{-2})	Anthocyanin (μg cm^{-2})	Mass/area (mg cm^{-2})	Nitrogen (%)	Phosphorus (%)	Photosynthetic efficiency (*Fv*/*F*m)
Anthocyanic								
Acer rubrum								
Mature (mean ± SE)	32.05 ± 7.82	2.69 ± 0.08	2.80 ± 0.61		6.86 ± 1.32	1.65 ± 0.32	0.24 ± 0.06	0.806 ± 0.014
Senescent (mean ± SE)	2.54 ± 1.36	1.95 ± 0.41	1.34 ± 0.24	5.7 ± 2.0	5.71 ± 1.27	0.61 ± 0.16	0.07 ± 0.02	0.382 ± 0.195
Acer saccharum								
Mature (mean ± SE)	32.51 ± 10.99	2.72 ± 0.06	2.96 ± 0.73		4.97 ± 0.62	1.94 ± 0.13	0.26 ± 0.03	0.776 ± 0.028
Senescent (mean ± SE)	1.69 ± 0.97	2.14 ± 0.87	1.26 ± 0.49	2.0 ± 0.6	5.14 ± 0.90	0.85 ± 0.19	0.19 ± 0.07	0.343 ± 0.240
Cornus alternifolia								
Mature (mean ± SE)	26.65 ± 2.59	2.78 ± 0.24	2.31 ± 0.45		4.56 ± 0.45	1.52 ± 0.39	0.23 ± 0.09	0.792 ± 0.014
Senescent (mean ± SE)	1.26 ± 0.91	1.95 ± 1.10	0.83 ± 0.37	3.0 ± 1.9	4.58 ± 0.82	0.65 ± 0.09	0.12 ± 0.09	0.376 ± 0.018
Fraxinus americana								
Mature (mean ± SE)	25.68 ± 6.28	3.20 ± 0.31	2.80 ± 0.48		6.58 ± 0.45	2.25 ± 0.07	0.18 ± 0.04	0.804 ± 0.016
Senescent (mean ± SE)	3.25 ± 1.71	2.14 ± 0.39	1.52 ± 0.68	2.2 ± 0.7	5.91 ± 1.10	1.10 ± 0.23	0.17 ± 0.09	0.092 ± 0.099
Prunus serotina								
Mature (mean ± SE)	30.83 ± 7.24	2.64 ± 0.10	2.42 ± 0.62		5.14 ± 1.01	1.89 ± 0.56	0.41 ± 0.13	0.805 ± 0.020
Senescent (mean ± SE)	1.25 ± 1.42	2.71 ± 0.46	0.68 ± 0.40	1.5 ± 0.4	4.38 ± 0.86	0.74 ± 0.12	0.27 ± 0.10	0.277 ± 0.290
Quercus rubra								
Mature (mean ± SE)	40.26 ± 6.23	2.78 ± 0.20	3.36 ± 0.46		6.81 ± 1.46	2.32 ± 0.12	0.16 ± 0.02	0.813 ± 0.028
Senescent (mean ± SE)	1.65 ± 0.69	1.96 ± 0.56	1.95 ± 0.50	3.0 ± 0.8	5.97 ± 1.03	0.83 ± 0.07	0.10 ± 0.04	0.233 ± 0.206
Vaccinium corymbosum								
Mature (mean ± SE)	40.17 ± 7.56	2.70 ± 0.05	3.01 ± 0.44		6.78 ± 0.68	1.52 ± 0.18	0.07 ± 0.01	0.814 ± 0.016
Senescent (mean ± SE)	3.64 ± 2.51	2.28 ± 0.49	1.13 ± 0.44	5.2 ± 3.3	6.12 ± 0.26	0.69 ± 0.04	0.04 ± 0.00	0.186 ± 0.169
Viburnum alnifolium								
Mature (mean ± SE)	10.16 ± 3.69	2.74 ± 0.09	1.55 ± 0.30		3.04 ± 0.23	1.73 ± 0.33	0.14 ± 0.03	0.776 ± 0.043
Senescent (mean ± SE)	1.28 ± 0.99	2.21 ± 0.23	0.86 ± 0.36	1.2 ± 0.4	2.62 ± 0.52	0.81 ± 0.09	0.09 ± 0.04	0.246 ± 0.184

Table 1 *Continued*

Species/Stage	Chlorophyll (μg cm^{-2})	(*a*/*b*)	Carotenoids (μg cm^{-2})	Anthocyanin (μg cm^{-2})	Mass/area (mg cm^{-2})	Nitrogen (%)	Phosphorus (%)	Photosynthetic efficiency (*Fv*/*F*m)
Viburnum cassinoides								
Mature (mean ± SE)	17.89 ± 2.68	2.73 ± 0.14	1.72 ± 0.21		6.90 ± 0.35	1.10 ± 0.12	0.13 ± 0.02	0.717 ± 0.017
Senescent (mean ± SE)	2.71 ± 1.74	1.74 ± 0.27	0.96 ± 0.36	2.7 ± 0.5	5.49 ± 0.48	0.49 ± 0.02	0.06 ± 0.01	0.078 ± 0.079
Non-anthocyanic								
Acer pensylvanicum								
Mature (mean ± SE)	24.87 ± 7.01	2.35 ± 0.45	1.82 ± 0.47		5.49 ± 0.48	0.49 ± 0.02	0.06 ± 0.01	0.078 ± 0.079
Senescent (mean ± SE)	0.55 ± 0.56	2.52 ± 0.12	0.54 ± 0.23	<0.2	2.22 ± 0.19	0.47 ± 0.02	0.07 ± 0.03	0.308 ± 0.285
Betula alleghaniensis								
Mature (mean ± SE)					5.07 ± 0.41	2.02 ± 0.28	0.39 ± 0.09	
Senescent (mean ± SE)				<0.2	3.73 ± 0.68	0.90 ± 0.13	0.22 ± 0.04	
Betula populifolia								
Mature (mean ± SE)	27.70 ± 2.89	2.95 ± 0.22	2.58 ± 0.20		5.05 ± 0.45	2.34 ± 0.15	0.24 ± 0.06	0.817 ± 0.012
Senescent (mean ± SE)	1.92 ± 1.13	1.98 ± 0.62	1.46 ± 0.25	<0.2	4.74 ± 0.97	1.12 ± 0.13	0.17 ± 0.04	0.346 ± 0.255
Castanea dentata								
Mature (mean ± SE)	33.14 ± 7.58	2.44 ± 0.08	2.50 ± 0.53		3.95 ± 0.15	1.95 ± 0.31	0.16 ± 0.05	0.809 ± 0.008
Senescent (mean ± SE)	1.61 ± 1.11	2.30 ± 0.51	1.44 ± 0.30	<0.2	3.17 ± 0.27	0.79 ± 0.14	0.08 ± 0.02	0.157 ± 0.095
Fagus grandifolia								
Mature (mean ± SE)	29.30 ± 2.40	3.28 ± 0.25	2.00 ± 0.29		3.84 ± 0.94	2.30 ± 0.24	0.16 ± 0.04	0.808 ± 0.016
Senescent (mean ± SE)	1.17 ± 0.98	3.43 ± 0.96	0.92 ± 0.19	<0.2	2.51 ± 0.01	0.95 ± 0.24	0.11 ± 0.06	0.204 ± 0.072
Hamelia virginiana								
Mature (mean ± SE)	26.34 ± 2.88	2.52 ± 0.13	2.03 ± 0.15		4.25 ± 0.39	1.39 ± 0.12	0.12 ± 0.01	0.790 ± 0.024
Senescent (mean ± SE)	0.38 ± 0.32	2.68 ± 0.17	0.91 ± 0.42	<0.2	3.98 ± 0.54	0.61 ± 0.04	0.08 ± 0.02	0.262 ± 0.209
Ilex verticillata								
Mature (mean ± SE)	28.86 ± 6.05	2.94 ± 0.17	3.11 ± 0.54		4.60 ± 0.56	2.09 ± 0.26	0.15 ± 0.01	0.799 ± 0.011
Senescent (mean ± SE)	3.32 ± 2.59	2.67 ± 0.17	2.47 ± 1.19	0.9 ± 0.2	3.89 ± 0.12	1.55 ± 0.17	0.09 ± 0.00	0.101 ± 0.046

Table 1 *Continued*

Species/Stage	Chlorophyll (μg cm^{-2})	(*a*/*b*)	Carotenoids (μg cm^{-2})	Anthocyanin (μg cm^{-2})	Mass/area (mg cm^{-2})	Nitrogen (%)	Phosphorus (%)	Photosynthetic efficiency (*Fv*/*F*m)
Populus grandidentata								
Mature (mean ± SE)	36.19 ± 8.81	2.70 ± 0.14	2.79 ± 0.48		5.10 ± 0.20	2.19 ± 0.36	0.15 ± 0.02	0.814 ± 0.012
Senescent (mean ± SE)						0.94 ± 0.06	0.10 ± 0.01	
Populus tremuloides								
Mature (mean ± SE)	36.38 ± 11.57	2.98 ± 0.11	3.26 ± 0.75		7.89 ± 1.30	2.15 ± 0.41	0.20 ± 0.10	0.834 ± 0.005
Senescent (mean ± SE)	3.00 ± 1.93	2.56 ± 0.29	1.84 ± 0.42	<0.5	6.73 ± 0.91	1.16 ± 0.10	0.12 ± 0.02	0.410 ± 0.115
Summaries								
Mature								
Anthocyanic (mean ± SE)	28.47 ± 9.80	2.78 ± 0.17	2.55 ± 0.60	<0.2	5.74 ± 1.38	1.77 ± 0.38	0.20 ± 0.10	0.789 ± 0.031
Non-anthocyanic (mean ± SE)	29.10 ± 5.89	2.77 ± 0.32	2.51 ± 0.53	<0.2	4.73 ± 1.40	2.01 ± 0.31	0.20 ± 0.08	0.809 ± 0.013
Senescent								
Anthocyanic (mean ± SE)	2.14 ± 0.92	2.12 ± 0.44	1.17 ± 0.40	2.9 ± 1.5	5.10 ± 1.11	0.75 ± 0.17	0.12 ± 0.07	0.239 ± 0.111
Non-anthocyanic (mean ± SE)	1.71 ± 1.13	2.59 ± 0.44	1.09 ± 0.50	<0.3	3.87 ± 1.42	0.94 ± 0.32	0.12 ± 0.05	0.255 ± 0.109
Significance								
Mature (*P*)	0.500 NS	0.500 NS	0.443 NS	NS	0.075 NS	0.094 NS	0.482 NS	0.084 NS
Senescent (*P*)	0.205 NS	0.021*	0.377 MS	0.002**	0.040*	0.084 NS	0.482 NS	0.378 NS

[†]Values are the means of two leaves selected from three individuals, $n = 6$; *Fv*/*F*m values are the means of two leaves selected from three individuals, $n = 6$; nutrient values are the means of three pooled samples (at least six leaves) from each individual, $n = 3$.

(NS), not significant.

Timing of leaf senescence

The phenologies of 16 species (the 18 analyzed for pigment changes minus *Populus grandidentata* and *Populus tremuloides*; Table 1) were followed from weekly observations of three tagged individuals at the Prospect Hill Tract. Percentages of leaves present, as well as leaf color, were assessed visually in relation to full foliage production from 1991 to 1999 (except for in 1992). We assessed the influence of irradiance and quantum ratios of red : far-red bandwidths (R : FR; Lee *et al*. 1996) on pigment changes and senescence of six species: *Acer rubrum*, *Acer saccharum*, *Cornus alternifolia*, *Fagus grandifolia*, *Quercus rubra* and *Viburnum alnifolium*. Three leaves of three individuals of each species from branches growing in partial shade with some exposure to direct sunlight were exposed in situ to five treatments: high irradiance and high R : FR; medium irradiance and high R : FR; medium irradiance and low R : FR; low irradiance and high R : FR; and low irradiance and low R : FR. Shade conditions were produced with combinations of energy films (3M, St Paul, MN, USA; Lee *et al*. 1996), with the spectral qualities of sunlight (R : FR of 1.15) and forest shade (R : FR of 0.25), and shade cloth. Irradiance was measured with a Li-190 quantum sensor (Li-Cor, Lincoln, Nebraska, USA; measuring photosynthetically active radiation [PAR], 400–700 nm) and a Li-1400 datalogger (Li-Cor). Changes in R : FR by the treatments were documented with a Li-1800 spectroradiometer (Li-Cor). The control (high irradiance) treatment was 92.4% of solar PAR; the medium irradiance treatments were 18% of solar PAR; and the low irradiance treatments were 3% of solar PAR. We gently stapled 2 cm × 6 cm 'sandwiches' of the appropriate combination of materials onto leaves on 21 August 1998, including a porous black fabric of 12% transparency on each undersurface. Changes in coloration of treated areas were qualitatively observed during the senescence process during September 1998–October 1998. Anthocyanin presence was graded on a 1–5 scale, where no evidence of anthocyanins was 1 and bright red leaves were 5. Chlorophyll breakdown was graded on a 1–5 scale, where normal green appearance was 1 and complete loss of green color was 5.

Leaf temperature measurements

We assessed the potential effect of anthocyanins on leaf temperature by comparing red and green leaves of *Vaccinium corymbosum* (n = 60 of each) and *Quercus rubra* (n = 40 of each) to direct sunlight in ambient outdoor temperatures on sunny (approximately 1500 μmol m^{-2} s^{-1}, 400–700 nm PAR) and cool (approximately 8°C) days from 24 October 1998 to 26 October 1998. We exposed freshly picked branches, with their ends cut and immersed in water, to direct sunlight for 30 min. As no leaves increased rapidly and steadily in temperature, we deduced that our immersion technique prevented the formation of embolisms. Leaf temperatures were measured with an infrared telethermometer (model OS-500; Omega Engineering, Stamford, CT, USA).

Statistical analyses

We compared average measurements of the nine yellow- and red-senescing species by Student's t-test. We analyzed relations among variables, measured from three individuals of the 18 species, with Pearson product correlations (SPSS for Windows, release 10.07; SPSS, Chicago, IL, USA). We also compared temperatures of the red- and green-senescing leaves by Student's t-test. Chlorophyll concentration was an indication of senescence. Because changes in senescing leaves were more pronounced when chlorophyll concentrations were low, we plotted xanthophyll and anthocyanin concentrations, and Fv/Fm against the log of chlorophyll concentrations. The relations between Fv/Fm and log chlorophyll (as the dependent variable) among species and pigment colors were compared as a mixed-model ANCOVA (SAS, version 9; SAS Institute, Cary, NC, USA).

RESULTS

All of the 89 observed species changed color from green to yellow, brown or red during senescence (Appendix I). Many species (62 species or 70%) produced anthocyanins during senescence. Although most became a shade of red, some appeared light- to dark-brown because of the presence of chlorophyll and anthocyanin, although the

brown color in some species without anthocyanins was the result of an accumulation of tannins. Certain families (e.g. Caprifoliaceae, Cornaceae and Rosaceae) were represented by many anthocyanic species; some (e.g. Ericaceae and Fagaceae) had species with and without anthocyanins; and some (e.g. Betulaceae) were without anthocyanins.

Yellow-turning leaves produced color by the retention of xanthophylls in senescing chloroplasts of mesophyll cells. Leaves senescing some shade of red produced color from the accumulation of anthocyanins in the vacuoles of different cell layers. The most frequently pigmented cell layer in all but two species was the palisade parenchyma (Appendix I). Thirteen species produced anthocyanins in the epidermis (but only two species exclusively so). No anthocyanins were observable in mature green leaves by these microscopic observations.

We compared nine of the more common yellow-senescing taxa with nine of the more common red-senescing taxa at Harvard Forest. Chlorophyll contents varied among mature leaves of the 18 taxa, with a mean of 29 μg cm^{-2}, and a range of 10–40 μg cm^{-2}. Chlorophyll contents were dramatically reduced during senescence, and did not vary between the anthocyanic and non-anthocyanic taxa (Table 1). Chlorophyll *a*/*b* ratios were similar in mature leaves of both groups, but were significantly reduced in the anthocyanic species late in senescence (Table 1). Red species produced significantly higher quantities of anthocyanins during senescence, and these compounds were not detectable by absorbance of extracts at 525 nm in normal green leaves (Table 1). Anthocyanins were detected when chlorophyll levels dropped below 20 μg cm^{-2} (Fig. 1). Total xanthophyll concentrations did not differ between these two species groups (Table 1; Fig. 2).

The senescent leaves of anthocyanic species were significantly higher in mass/area and lower in chlorophyll *a*/*b* than the non-anthocyanic species (Table 1). Trends towards reduced nitrogen content in the mature and senescent anthocyanic leaves were not significant ($P = 0.094$ and $P = 0.084$, respectively). Phosphorus levels were similar between anthocyanic and non-anthocyanic leaves. Early morning dark-acclimated *Fv*/*F*m declined to a similar extent in senescent leaves of both groups (Table 1; Fig. 3). The slopes of the lines in Fig. 3 between the red- and yellow-leaved species were not significantly different, although some species were different from others.

Anthocyanin concentrations of senescent leaves significantly correlated to four variables: greater mass/area; less nitrogen in senescent leaves and less nitrogen in mature leaves; and carbon : nitrogen ratios in senescent leaves (Table 2). Other variables were significantly correlated as chlorophyll and nitrogen contents per area in senescent leaves. Nitrogen resorption efficiency did not correlate to anthocyanin content, but correlated negatively to

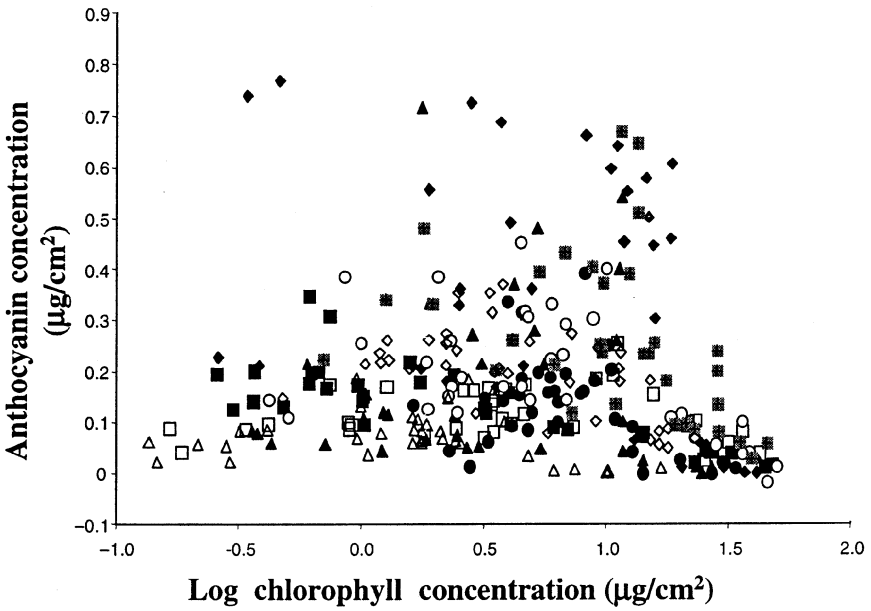

Fig. 1 Log chlorophyll concentrations plotted against anthocyanin concentrations in leaves of red-senescing species at Harvard Forest.

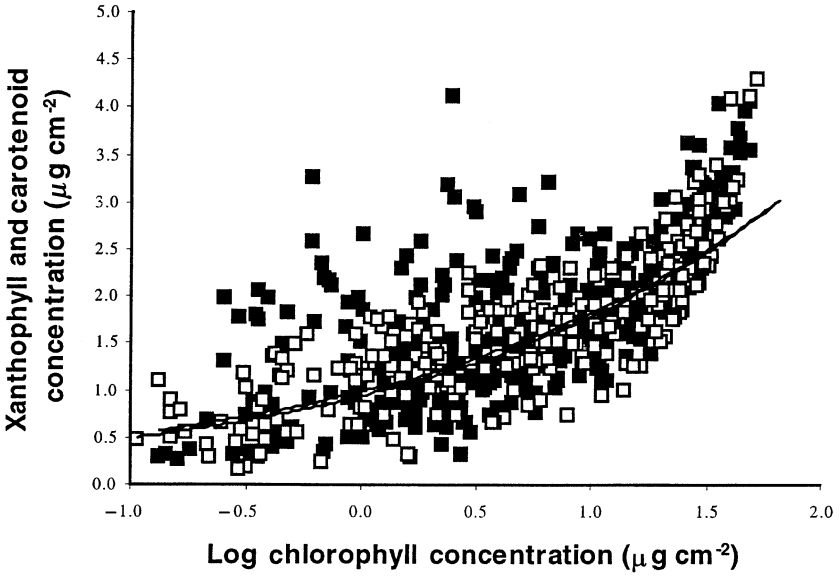

Fig. 2 Log chlorophyll concentrations plotted against total xanthophyll concentrations in leaves of red- and yellow-senescing species at Harvard Forest. Points were fitted to an exponential function, and the curves were virtually identical between the two groups: yellow-senescing species, $y = 0.983 \times 10^{0.269x}$, $r^2 = 0.441$; red-senescing species, $y = 0.938 \times 10^{0.277x}$, $r^2 = 0.584$.

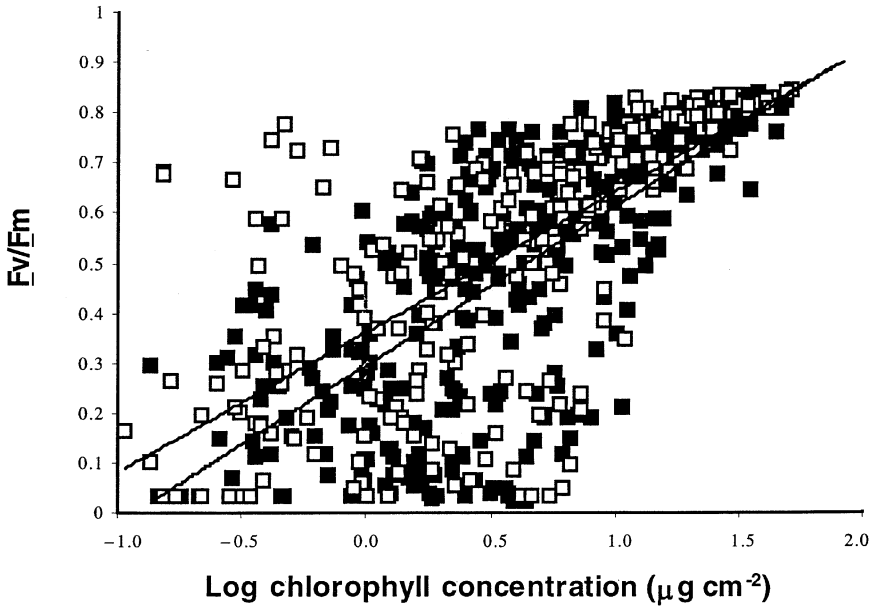

Fig. 3 Log chlorophyll concentrations plotted against dark-acclimated photosynthetic efficiency (*Fv*/*F*m) in leaves of red- and yellow-senescing species at Harvard Forest. The slopes of the two lines were not significantly different: yellow-senescing species, $y = 0.280x + 0.362$, $r^2 = 0.491$; red-senescing species, $y = 0.316x + 0.296$, $r^2 = 0.536$.

the chlorophyll content of senescent leaves. Photosynthetic efficiency in senescent leaves only correlated negatively to the nitrogen : phosphorus ratio in senescent leaves.

Leaf-fall was quite consistent from year to year (Table 3). Some species lost leaves before other species, and this order was generally repeated each year. The mean day for 50% leaf-fall for all species varied from 287 to 294 Julian days. The intensity of color production varied from year to year (J. O'Keefe, unpubl. obs., 1991–2002) – a combination of the number of leaves left and the intensity of pigmentation in those leaves.

Shading affected the intensity of color production and rate of senescence in all five species examined (Table 4). However, all four shade treatments, high and low R : FR, and 3% and 18% sunlight, had similar effects. Shading suppressed chlorophyll loss and anthocyanin production in the four species that produced red-senescent leaves. In the one yellow-senescing species, *Fagus grandifolia*, shading accelerated the rate of chlorophyll loss.

Table 2 Pearson product correlations between measurements of leaf samples of individual plants of the 18 species studied

Measurement	Chlorophyll (mature) (mg cm^{-2})	Chlorophyll (senescent) (mg cm^{-2})	Photosynthetic efficiency (senescent) (*Fv*/*F*m)	Leaf mass (senescent) (mg cm^{-2})	Nitrogen (senescent) (%)	Nitrogen (mature) (%)	Nitrogen per area (senescent) (μg cm^{-2})	Nitrogen resorption (senescent) (%)	Carbon : Nitrogen (senescent)	Nitrogen : Phosphorus (senescent)	Phosphorus (senescent) (%)
Anthocyanins (μg cm^{-2})	0.168 NS	0.004 NS	0.022 NS	0.362**	−0.334*	−0.361**	−0.052 NS	0.137 NS	0.420*	0.157 NS	−0.268 NS
Chlorophyll (mature) (mg cm^{-2})		0.237 NS	0.111 NS	0.404**	0.083 NS	0.308*	0.353*	0.153 NS	−0.125 NS	0.104 NS	0.110 NS
Chlorophyll (senescent) (mg cm^{-2})			0.062 NS	0.250 NS	0.432**	0.202 NS	0.481**	−0.368*	−0.335 NS	0.325*	−0.073 NS
Photosynthetic efficiency (senescent) (*Fv*/*F*m)				0.145 NS	−0.076 NS	0.029 NS	0.078 NS	0.122 NS	−0.010 NS	−0.291*	0.112 NS
Leaf mass (senescent) (Mg cm^{-2})					0.067 NS	0.105 NS	0.741**	−0.017 NS	−0.048 NS	−0.016 NS	−0.014 NS
Nitrogen (senescent) (%)						0.598**	0.691**	−0.748**	0.917**	0.250 NS	0.227 NS
Nitrogen (mature) (%)							0.495** **	0.068 NS	−0.672**	0.036 NS	0.280 NS
Nitrogen per area (senescent) (μg cm^{-2})								−0.463**	0.641**	0.157 NS	0.125 NS
Nitrogen resorption (senescent) (%)									0.620**	−0.281NS	−0.069 NS
Carbon : Nitrogen (senescent)										−0.172 NS	−0.316*
Phosphorus (senescent) (%)											−0.729**

*$P < 0.05$; **$P = 0.005$.
(NS), not significant.

Table 3 Estimates of 50% crown foliage cover (Julian Day) for three tagged trees of each of 16 species at Harvard Forest, based on weekly visits since 1991 (except for 1992)

	Year								
Species	1991	1993	1994	1995	1996	1997	1998	1999	Mean ± SD
Anthocyanic									
Acer rubrum	279	278	279	276	281	282	281	286	280 ± 3
Acer saccharum	296	292	290	291	293	296	290	294	293 ± 2
Cornus alternifolia	288	286	289	283	290	289	284	290	287 ± 3
Fraxinus americana	283	288	280	282	289	290	287	283	285 ± 4
Prunus serotina	293	291	291	287	288	290	283	292	290 ± 3
Quercus rubra	297	302	296	296	296	305	296	301	299 ± 4
Vaccinium corymbosum	293	295	292	293	295	303	298	298	296 ± 4
Viburnum alnifolium	290	292	286	276	291	289	290	288	288 ± 6
Viburnum cassinoides	298	293	298	282	300	306	301	303	298 ± 7
Non-anthocyanic									
Acer pensylvanicum	288	292	289	282	289	285	290	293	288 ± 5
Betula alleghaniensis	279	279	278	277	285	283	281	281	281 ± 4
Betula populifolia	281	280	284	281	286	288	283	286	284 ± 4
Castanea dentata	289	291	297	295	294	296	292	291	293 ± 4
Fagus grandifolia	303	310	306	308	304	301	306	307	306 ± 3
Hamamelis virginiana	286	288	285	285	288	289	288	290	287 ± 4
Ilex verticillata	300	298	304	302	303	309	309	305	304 ± 4
Populus grandidentata	–	–	–	–	–	–	–	–	–
Populus tremuloides	–	–	–	–	–	–	–	–	–
Year summaries									
Mean	290	291	290	287	292	294	289	294	290 ± 6
SD	7	8	8	9	6	9	9	7	

Table 4 Effects of artificial shade treatments and clear plastic control on anthocyanin production and chlorophyll loss during senescence†

Taxon	HRR	LFR	LRR	MFR	MRR
Anthocyanin production					
Acer saccharum	5	1	2	2	1
Acer rubrum	5	2	2	2	2
Cornus alternifolia	5	1	1	1	1
Quercus rubra	5	1	2	1	1
Viburnum alnifolium	5	1	1	1	1
Chlorophyll breakdown					
Acer rubrum	5	2	2	2	2
Acer saccharum	4	3	4	3	2
Cornus alternifolia	4	2	2	2	2
Quercus rubra	4	3	3	3	3
Viburnum alnifolium	4	2	2	3	3
Fagus grandifolia	3	4	4	4	4

†Artificial shade treatments: 3% (L) and 18% (M) of full solar photosynthetically active radiation (PAR), and quantum ratios of red (R) and far-eyed bandwidths (FR). R : FR of 0.25 (FR) and 1.15 (RR); clear plastic control: HRR (85 % PAR and 1.15 R : FR); values are 0–5 from least to maximum pigment present.

The temperatures of anthocyanic-senescent leaves were not significantly higher in sunlight than green leaves in both *Quercus rubra* (24.1 ± 0.7°C (SE) green vs 23.8 ± 1.1°C red) and *Vaccinium corymbosum* (19.5 ± 0.7°C green vs 19.9 ± 0.6°C red), but leaf temperatures were increased in sunlight compared with leaf temperatures in shade.

DISCUSSION

Patterns of autumnal coloration

Leaves of the red-senescing (anthocyanic) and yellow-senescing (non-anthocyanic) species in the present comparative study changed both similarly and differently. Total xanthophyll concentrations steadily declined in all 18 species during leaf senescence (Fig. 2), not differing between the yellow- and red-senescing taxa and strongly correlated to chlorophyll breakdown in both groups. Given the association of xanthophylls with the light-harvesting chlorophyll-binding proteins (Taiz & Zeiger 1998), such a correlation is not surprising. Declines in xanthophyll concentrations might be associated with shifts in individual pigments during senescence (Goodwin 1958; Sanger 1971), but this was not assessed in the present research.

Anthocyanins were not present in mature leaves of the 62 taxa we observed that produced anthocyanins in different tissues (Appendix I). Anthocyanins, which were easily observable in the large central vacuoles, almost always accumulated in the mesophyll and usually in the palisade parenchyma layer. Anthocyanins were not present at detectable levels in mature leaves of the nine red-senescing species whose pigments we monitored. Clearly, as previous authors have also shown, these pigments are not present and subsequently unmasked in mature tissues during senescence (Sanger 1971; Chang *et al*. 1989). Instead, they are produced in mid senescence, when leaves generally have less than 20 μg cm^{-2} of chlorophyll.

Shading affected both the timing of senescence and the rate of anthocyanin accumulation during senescence, but reduced R : FR did not affect these changes (Table 4). Reduced R : FR, affecting phytochrome equilibrium, promoted leaf senescence and anthocyanin production in several species (Guiamet *et al*. 1989; Nooden *et al*. 1996; Rousseaux *et al*. 1996; Gan & Amasino 1997), but at much lower R : FR than in the present study. In all but one species (*Fagus grandifolia*), shading slowed the rate of senescence and anthocyanin production. Cold and bright days during autumn are known to intensify color production (King 1997; Kozlowski & Pallardy 1997).

Although environmental factors influence the intensity in color of autumn leaf senescence, the timing of leaf senescence was remarkably uniform among species from year to year (Table 3). Certain species underwent senescence earlier than others from year to year, but their date of 50% leaf loss varied little over the 8 years observed in the present research. *Acer rubrum*, on 8 October, was the earliest species with 50% leaf-fall, followed by *Betula allegheniensis* (9 October), *Betula populifolia* (12 October) and *Fraxinus americana* (13 October). The latest senescing species was *Fagus grandifolia* (2 November, many of whose brown leaves persist on trees well into the winter), preceded by *Ilex verticillata* (31 October, many of whose leaves fall partially green), *Quercus rubrum* (26 October) and *Viburnum cassinoides* (25 October). Thus, the timing of senescence, from earliest to latest species, averaged 3.5 weeks. In 2002, leaf-fall in these species was much later, perhaps affected by a higher September mean temperature (J. O'Keefe, unpubl. data, 2002).

Roles of anthocyanins during leaf senescence

Given the production and the active uptake of anthocyanins into vacuoles of leaf mesophyll cells, what might their function(s) be? The most reasonable explanation is that they are protection against the activation of chlorophyll breakdown products during senescence. During senescence, in the conversion of chloroplasts to gerontoplasts (Thomas 1997; Matile *et al*. 1999), photoreactive molecules could produce free radicals on exposure to bright light, possibly inactivating steps in the orderly breakdown of chlorophyll and the resorption of leaf nitrogen by stems (up to 90% of the retranslocated nitrogen; Smart 1994).

The maximum photosystem II quantum efficiency, as indicated by dark-adapted *Fv*/*F*m, declined gradually during senescence and then

rapidly towards the end of senescence in all species (Fig. 3). This slow decline suggests that the integrity of the light reaction complexes is maintained until near the end of senescence (Adams *et al*. 1990). Furthermore, the slope of those declines varied little between the red-senescing and the yellow-senescing species. However, dark-adapted *Fv*/*F*m was measured on leaves collected in predawn darkness, which may have had ample time to repair any damage from the previous day. Feild *et al*. (2001) showed that red leaves of *Cornus stolonifera* were less photo-inhibited and returned to original function more rapidly than senescent green leaves. Gould *et al*. (1995) argued that absorption by anthocyanins in leaves is well-placed to preferentially protect chlorophyll *b* rather than chlorophyll *a* molecules in the LHC-II complexes. Chlorophyll *a*/*b* has generally been shown to decline during senescence (Wolf 1956; Adams *et al*. 1990; Zhu & Wild 1995), but Dean *et al*. (1993) showed an increase for *Populus tremuloides*. The nine anthocyanic taxa in the present study had lower chlorophyll *a*/*b* than the nine yellow-senescing species (Table 1). However, chlorophyll *a* is the final form before breakdown (Matile *et al*. 1999) and the significance of this difference needs further investigation.

If anthocyanins protect senescing leaves and allow a more efficient or complete resorption of nutrients, then these leaves should have lower nutrient contents compared with the yellow-senescing species. Collectively, senescent leaves of the anthocyanic species retained less nitrogen than the non-anthocyanic species, but not significantly so ($P = 0.084$; Table 1), and the phosphorus concentrations did not differ. The same trend was seen in mature leaves. Senescent leaves of the anthocyanic taxa were significantly greater in mass/area. Nitrogen resorption efficiencies were not significantly higher (Killingbeck 1996). However, this calculation requires accurate estimations of nitrogen contents in leaves at maturity and at the time of leaf-fall. Because the chlorophyll contents of leaves in the present study were low compared with normally measured levels of approximately 40 μg cm^{-2} (Björkman 1981; Lee *et al*. 1990), and chlorophyll accounts for much of the nitrogen present in leaves, these calculations would give inaccurately high resorption efficiencies. Differences in these values would likely be a result of early rates of senescence and not a result of efficiencies of nitrogen use. Killingbeck (1996) also recommended the use of the nutrient content of senescent leaves (nutrient resorption proficiency). The concentrations of nitrogen in senescent leaves of the non-anthocyanic species were similar to those reviewed by Aerts (1996) and Killingbeck (1996), and those of the anthocyanic species were only slightly lower. However, comparisons of these variables among pooled leaves of three individual plants from each of the 18 species, varying in anthocyanin content, revealed that higher anthocyanin contents were associated with lower nitrogen concentrations in senescent and mature leaves (Table 2). Anthocyanin content of senescent leaves significantly negatively correlated to nitrogen in both mature and senescent leaves. This anthocyanin content also correlated to the mass/area of both mature and senescent leaves, and thus highly and negatively correlated to the carbon : nitrogen ratio of senescent leaves. Anthocyanin content did not correlate to nitrogen in senescent leaves on a per area basis.

Although the negative correlation between nitrogen concentrations and anthocyanin levels in leaves was consistent with the hypothesis of photoprotection, this hypothesis fails to explain the greater leaf mass/area in both mature and senescent leaves, and the lower nitrogen contents of these leaves (compared to those of the yellow-senescing species) prior to senescence. Because the nitrogen contents in leaves of species can vary with site and between years (Killingbeck 1996), and the differences in nitrogen contents do not have to be large to be biologically significant, it might be difficult to demonstrate this relation clearly. Perhaps field experiments comparing wild and anthocyanin-free mutants would be the best approach.

Hoch *et al*. (2001) argued that photoprotection would be more important in species with lower maximum photosynthesis, such as shade-tolerant or late successional species. The species in the sample in the present study varied in successional status in both groups, although some yellow-senescing species were among the most shade tolerant (*Hamamelis virginiana* and *Acer pensylvanicum*). This relation should be explored in a much larger sample of individual trees within species, and in many more species that undergo senescence

both with and without the production of anthocyanins.

An alternative hypothesis is that anthocyanins influence leaf senescence by elevating leaf temperature. We did not find that anthocyanic leaves had increased temperatures in two species, *Quercus rubra* and *Vaccinium corymbosum.* These results are inconsistent with classical evidence for such a correlation (Smith 1909; Lee *et al*. 1987).

An additional hypothesis is that anthocyanins mediate biological interactions. Stiles (1982) argued that red leaves could attract frugivorous birds as seed dispersers, and mentioned species in Rhus as examples; we observed anthocyanin production in five species of Rhus (Appendix I). Two species with the brightest red leaves (*Euonymus atropurpureus* and *Parthenocissus quinquefolia*) might also be candidates for such a mechanism, but almost all of the species senesce in the absence of dispersable fruits. Hamilton and Brown (2001) argued that color production could reduce herbivory from aphids by reducing egg-laying. Their evidence, based on literature surveys, was most significant for yellow coloration and only marginally so for anthocyanic coloration. As all species appear to retain xanthophylls to the same extent (Table 1; Fig. 2), and anthocyanins and residual chlorophyll mask yellow color production, we would thus expect strong selection pressure against anthocyanin production for protection against aphid damage, the opposite to their argument (see Lee 2002 for a more detailed analysis).

CONCLUSIONS

The majority of woody plants at the Harvard Forest site produce anthocyanins during senescence, even when leaves do not appear red. Our percentage of 70% of woody plants might be conservative, because the plants we observed as senescing yellow might produce anthocyanins in other parts of their distributions (Chang *et al*. 1989). Clearly, anthocyanins are synthesized de novo, midway in the leaf senescence process. The timing of senescence varied little from year to year. The decline in xanthophyll concentrations was identical in red- and yellow-senescing taxa. We found two traits significantly associated with anthocyanin production during senescence: lower chlorophyll *a*/*b* and greater leaf mass/area. These differences were detected in a sample of nine red-senescing and nine yellow-senescing taxa, among the most common participants in the show of autumn coloration in New England forests. The results only very weakly support a protective role of anthocyanins during senescence. Dark-acclimated *Fv*/*F*m declined the same in the two groups, although differences in reductions of this ratio could occur during daytime exposure to high light at low temperatures. Nitrogen contents of senescing leaves of anthocyanic species were slightly, but not significantly, lower (but so were those of mature leaves). However, anthocyanin content significantly negatively correlated to leaf nitrogen contents in senescent leaves. Clearly, more species should be surveyed to determine if these differences are wide spread. Finally, more detailed physiological studies, particularly of species with mutants differing in anthocyanin production, will add to our understanding of the functional role of anthocyanins during senescence.

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APPENDIX I

Anthocyanins present in tissue layers of senescing leaves

The numbers in parentheses indicate pigment production in increasing order of intensity, where 0 = no cells with anthocyanins present and 4 = all cells with intense coloration in vacuoles. The numbers refer to the following tissues in numerical order: adaxial epidermis, palisade parenchyma, spongy mesophyll, abaxial epidermis, trichomes/scales. Species with no numbers did not produce anthocyanins during senescence. The superscript numbers 1–8 indicate the locations of collection: [1]Prospect Hill Tract, Harvard Forest, Petersham, MA, 42°32′N, 72°11′W; [2]Harvard Pond, Petersham, MA, 42°30′N, 72°12′W; [3]Tully Pond, Royalston, MA, 42°38′N, 72°14′W; [4]O'Keefe property, Royalston, MA, 42°40′N, 72°16′W; [5]Wickett Pond, Wendell, MA, 42°33′N, 72°26′W; [6]Spirit Falls, Royalston, MA, 42°40′N, 72°12′W; [7]Sentinel Elm Farm, Orange, MA, 42°36′N, 72°15′W; [8]Barton's Cove, Gill, MA, 42°36′N, 72°31′W. All locations were at 350–400 m above sea level, except for Barton's Cove which was at 50 m above sea level. [9]Species is a naturalized exotic.

Aceraceae: *Acer pensylvanicum* L.[1]; *Acer rubrum* L.[1] (14110); *Acer saccharum* H. Marsh.[1] (03000); *Acer spicatum* Lam.[1] (32000).

Anacardiaceae: *Rhus copallina* L.[1] (04100); *Rhus glabra* L.[5] (04010); *Rhus radicans* L.[1] (04100); *Rhus typhina* L.[1] (04000); *Rhus vernix* L.[3] (04000).

Aquifoliaceae: *Ilex verticillata* (L.) A. Gray[1] (02000); *Nemopanthus mucronata* (L.) Trel.[2] (03000).

Araliaceae: *Aralia nudicaulis* L.[1] (02100); *Aralia spinosa* L.[1] (03100).

Berberidaceae: *Berberis vulgaris* L.[5] (03010).

Betulaceae: *Alnus rugosa* (Duroi) Spreng.[1] (03000); *Betula alleghaniensis* Britton[1]; *Betula lenta* L.[1]; *Betula papyrifera* H. Marsh[1]; *Betula populifolia* H. Marsh[1]; *Corylus americana* Walt.[3]; *Corylus cornuta* H. Marsh[3].

Caprifoliaceae: *Lonicera canadensis* H. Marsh[1] (03100); *Lonicera tatarica* L.[1,9] (04000); *Sambucus canadensis* L.[1]; *Sambucus racemosa* L.[1] (01000); *Viburnum acerifolium* L.[1] (04000); *Viburnum alnifolium* H. Marsh[1] (04000); *Viburnum cassinoides* L.[1] (04100); *Viburnum dentatum* L.[1] (03100).

Celastraceae: *Euonymus atropurporeus* Jacq.[1] (40000).

Cornaceae: *Cornus alternifolia* L. Fil.[1] (03000); *Cornus amomum* P. Mill.[1] (03000); *Cornus racemosa* Lam.[1] (03000); *Cornus stolonifera* Michx.[1] (04000).

Corylaceae: *Carpinus caroliniana* Walter[2] (03000).

Ericaceae: *Chamaedaphne calyculata* (L.) Muench[2] (04100); *Gaultheria procumbens* L.[1] (04000); *Kalmia angustifolia* L.[2] (03100); *Kalmia latifolia* L.[1]; *Lyonia ligustrina* (L.) DC.[1] (04000); *Rhododendron nudiflorum* (L.) J. Torr.[1]; *Vaccinium angustifolium* Ait.[5] (04000); *Vaccinium corymbosum* L.[1] (04000); *Vaccinium macrocarpon* Ait.[2] (04100).

Fabaceae: *Robinia pseudo-acacia* L.[1]

Fagaceae: *Castanea dentata* (H. Marsh) Borkh.[1]; *Fagus grandifolia* J. F. Ehrh.[1]; *Quercus alba* L.[1] (03200); *Quercus coccinea* Meunch[1] (03100); *Quercus rubra* L.[1] (03100).

Grossulariaceae: *Ribes sativum* Syme[1,9] (03000).

Hamamelidaceae: *Hamamelis virginiana* L.[1]

Hippocastanaceae: *Aesculus hippocastanum* L.[1,9]

Hypericaceae: *Hypericum boreale* (N. L. Britt.) Bickn.[5] (04110).

Juglandaceae: *Carya glabra* (P. Mill.) Sweet[7]; *Carya ovata* (P. Mill.) K. Koch[7]; *Juglans regia* L.[1]

Lauraceae: *Sassafras albicum* (Nutt.) C. Nees.[8] (04200).

Moraceae: *Morus rubra* L.[1]

Myricaceae: *Comptonia peregrina* (L.) Coult.[1] (44000); *Myrica gale* L.[3]

Nyssaceae: *Nyssa sylvatica* H. Marsh[1] (03000).

Oleaceae: *Fraxinus americana* L.[1] (04000).

Platanaceae: *Platanus occidentalis* L.[1]

Polygonaceae: *Polygonum articulata*[1,9] (12000).

Rhamnaceae: *Rhamnus cathartica* L.[1,9] (14110); *Rhamnus frangula* L.[1,9] (11120).

Rosaceae: *Amelanchier canadensis* (L.) Medic.[3] (03100); *Aronia arbutifolia* (L.) Ell.[1] (04100); *Aronia melanocarpa* (Michx.) Ell.[5] (03000); *Prunus pensylvanica* L. Fil.[1] (32000); *Prunus serotina* J. F. Ehrh.[1] (10000); *Prunus virginiana* L.[5] (33000); *Rosa multiflora* Thunb.[1,9] (22000); *Rubus allegheniensis* T. C. Porter[1] (04000); *Rubus hispidus* L.[5] (04000); *Rubus idaeus* L.[3] (22000); *Sorbus americana* H. Marsh.[1]; *Spirea latifolia* (Ait.) Borkh.[1] (03000); *Spirea tomentosa* L.[3]

Rubiaceae: *Cephalanthus occidentalis* L.[3] (03000).

Salicaceae: *Populus grandidentata* Michx.[1] (03000); *Populus tremuloides* Michx.[1]; *Salix babylonica* L.[1,9]; *Salix* cf. *purporea*[3] (03000).

Tiliaceae: *Tilia americana* L.[4]

Ulmaceae: *Ulmus americana* L.[1] (01000).

Vitaceae: *Parthenocissus quinquefolia* (L.) Planch.[1] (14000); *Vitis labrusca* L.[1]