Why Leaves Turn Red

Pigments called anthocyanins probably protect leaves from light damage by direct shielding and by scavenging free radicals

David W. Lee and Kevin S. Gould

Many forests, like those spread throughout New England, have just changed color in a spectacular way, as they do each fall. The phenomenon is familiar as well as dramatic, yet why it should happen has been a long-standing enigma. When we were in school, the standard textbooks said that foliage changes color because the breakdown of green chlorophyll molecules unmasks other pigments, like the yellow-to-orange xanthophylls and the red-or-blue anthocyanins, which, we were told, serve no particular function during the autumn senescence of leaves. Now botanists know better.

Indeed, a completely new appreciation for these colorful pigments has developed over the past decade or so, in part from our studies of trees in the Harvard Forest, a nature sanctuary in central Massachusetts maintained for scientific research. There, during September and October, one sees the leaves on dozens of woody species changing color. In some plants, such as the witch hazel (Hamamelis virginiana), it is indeed the loss of chlorophyll that reveals yellow carotenoid pigments, just as the textbooks say. However, for the forest's 70 percent of tree species that contain anthocyanin pigments (which produce colors ranging from brown to red, depending on how much chlorophyll the leaves retain), the story is quite different. For example, the brilliant fall foliage of the red oak (Quercus rubra) results from the accumulation of anthocyanin in the vacuoles (large, fluid-filled cavities) of cells lying just under the leaves' upper epidermis layer.

Anthocyanins are elaborate pigment molecules, widespread among land plants. They account not only for the autumn hues of temperate woodlands, but also for the flushes of developing red foliage seen in tropical forests, on the undersurface of shaded leaves and in crop plants suffering drought or nutrient deficiency. But plants can also have other red pigments. Carotenoids, often rhodoxanthin, produce red color in the senescing leaves of some conifers as well as in the common box (Buxus sempervirens), which decorates many suburban lawns. Betalain pigments color leaves red in a single order of flowering plants, and a few other miscellaneous pigments produce burgundy hues in very rare cases. But of all the red pigments, the anthocyanins are the most widespread.

We have collaborated in studying anthocyanin pigments since 1993 and are beginning to develop some working hypotheses about their function. It's curious that an understanding has been so long in coming, given the fact these red pigments have been subjected to scientific scrutiny for nearly 200 years.

The Discovery of Anthocyanins

Anthocyanins had been observed for centuries as "colored cell sap." In 1835 the German botanist Ludwig Marquart gave them their name, deriving anthocyanin from the Greek anthos, meaning flower, and kyanos, meaning blue. Many long-standing misconceptions about anthocyanin function date from these early observations, notably that these pigments arise from the breakdown of chlorophyll during autumn.

Given how striking and attractive red foliage is, it may seem baffling that botanists remained ignorant about the phenomenon for so long. There are various reasons for this. First, because anthocyanins are responsible for the colors of fruits and flowers as well as of leaves, it was natural to concentrate on pigmentation in the former economically important organs, for which the function of anthocyanin seems obvious—to attract animals for pollination and seed dispersal. Second, because the discoveries of Richard Willstätter and his colleagues about the molecular structure of anthocyanins from 1912 to 1916 were made shortly after the rediscovery of Mendel's laws of inheritance, the anthocyanins became an early subject of research in molecular genetics, rather than physiology. (Mendel's peas had distinctively colored flowers because of anthocyanins.) Third, the discovery that light can induce anthocyanin production inspired molecular biologists to study how light exposure activates genes involved in anthocyanin synthesis, again at the expense of research into anthocyanin function.

Botanists of the late 19th-century, most notably the Germans who studied plant anatomy and physiology, noticed that anthocyanin production rises when a plant is subjected to low temperatures and high light conditions. This observation led to the popular explanations that anthocyanins protect the photosynthetic

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Figure 1. Anthocyanins are common in the autumn leaves of mid-latitude trees, but these pigments also add flashes of red color to the foliage of tropical forests and to crop plants exposed to drought or nutrient deficiency. Occasionally, anthocyanins are present in leaves year-round, as in the variegated leaves of Horopito (Pseudowintera colorata), a common tree in New Zealand forests (above). The patterns of coloration in this species make the leaves ideal for studying the antioxidant properties of these intriguing molecules. (Except where noted, all photographs by the authors.)

structures against intense sunlight and help to warm leaves by increasing their rates of metabolism. These scientists lacked the instrumentation and detailed knowledge of photosynthesis to test their ideas. In the mid-20th century, investigators became aware that ultraviolet (UV) radiation could induce anthocyanin synthesis, leading to the hypothesis that anthocyanins protect plant tissues against UV damage. But, as it turns out, anthocyanins absorb rather weakly in the UV-B region of the spectrum (wavelengths of 285–320 nanometers), which is most responsible for damage to biological tissues; other colorless flavonoid pigments that are equally, or more, abundant in the leaves absorb UV-B much more strongly. Furthermore, anthocyanins are most commonly produced in the interiors of leaves and hence are poorly placed to protect leaves from the widespread effects of UV-B. These weaknesses were refuted by one of us (Lee) in 1987. So what good are anthocyanins to a leaf? Two recent discoveries have shed light on the mystery.

Red Sunscreen

When surroundings are bright and cold, photosynthetic efficiency often declines. The phenomenon, known as photoinhibition, has been attributed in part to impairment in one of the functional elements of photosynthesis. Normally, two units consisting of pigments, proteins and electron-transfer molecules—known as photosystems I and II—absorb light energy. Photoinhibition apparently involves a block in photosystem II. Unchecked, this impairment can permanently damage chloroplasts, cells and tissues.

Investigators can observe photoinhibition because when photosynthetic tissues receive a pulse of intense light, they immediately emit a pulse of visible light—that is, they fluoresce. Detailed analysis of this flash reveals much about photosynthetic function. New techniques to measure this fluorescence have helped investigators test the efficiency of the light reaction of photosynthesis under different conditions and to detect photoinhibition. A variety of factors can contribute to photoinhibition: intense sunlight; low temperature; acclimation of leaves to extreme shade with a subsequent exposure to high light; and inadequate phosphorus, which is important in the production of two energy-rich compounds crucial for photosynthesis—adenosine triphosphate (ATP) and the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH). When chloroplasts are overwhelmed with energy, the excess causes chemical and, ultimately, physical damage.

Plants have evolved several strategies to prevent photoinhibitory damage from intense light, in particular the interconversion of certain xanthophyll pigments as a way of quenching the overload of energy. Anthocyanins also efficiently protect against photoinhibition because they soak up radiant energy at wavelengths poorly absorbed by other accessory pigments, such as in the green waveband at around 530 nanometers. In intact tissues, the range of absorbance also extends into shorter

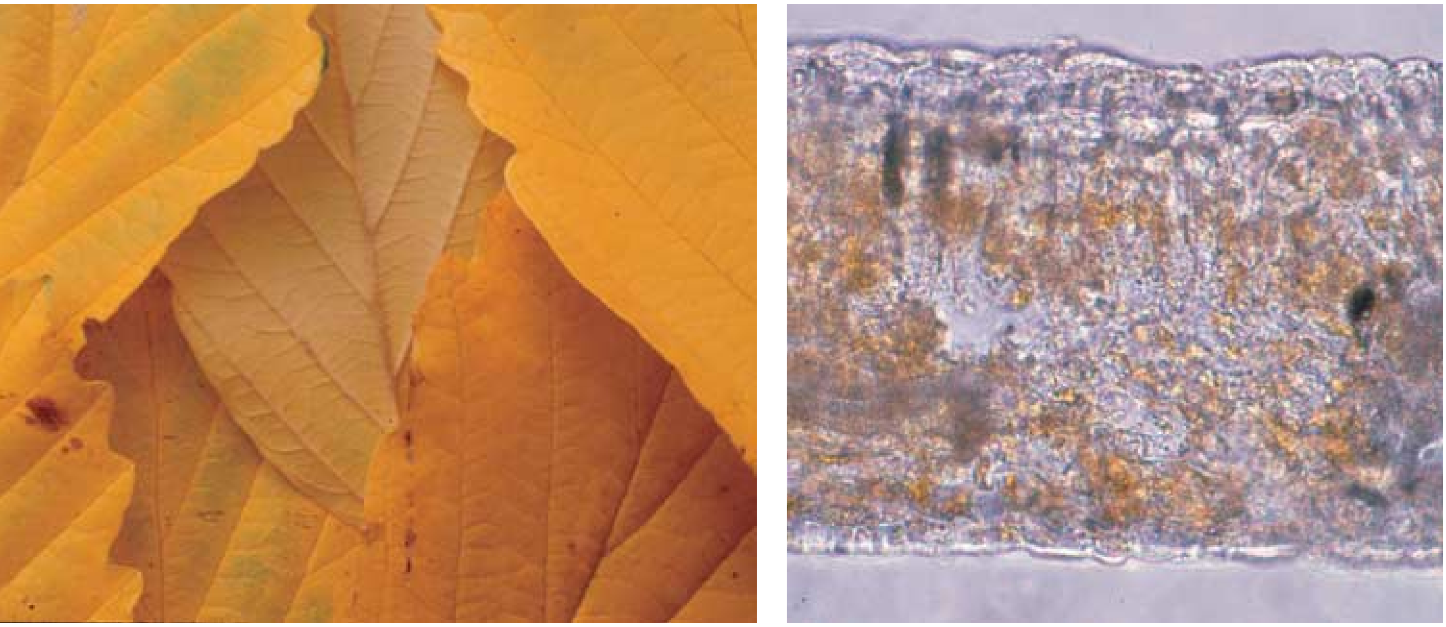

Figure 2. Some senescing autumn leaves, such as the witch hazel (Hamamelis virginiana, left) are yellow. A transverse section of a leaf (right) shows that the yellow pigments are clustered exclusively in the chloroplasts, which are degrading. The loss of chlorophyll reveals the yellow carotenoid pigments. (Except where noted, all microscopic images are magnified 250 times.)

(blue) wavelengths, overlapping with the absorbance of chlorophyll, particularly with chlorophyll b, one of the two major forms of the pigment. In addition, anthocyanins are very stable compounds in the mildly acidic environment of the cell vacuoles that contain them. The hardy anthocyanins can thus shield the more delicate chlorophyll molecules housed in the chloroplasts.

During the past decade fluorescence measurements have yielded growing evidence that the anthocyanins indeed protect against photoinhibition. Taylor Field, then a graduate student at Harvard University and now a faculty member at the University of Toronto; Noelle Holbrook at Harvard; and one of us (Lee) found support for the protective function of anthocyanins in the red-osier dogwood (Cornus stolonifera). We were able to compare the photosynthetic responses of senescing leaves that varied only in the presence or absence of anthocyanin near their surfaces. We hypothesized that this pigment layer would protect the photosynthetic tissues underneath. And we were right.

When exposed to intense light, the red leaves in our experimental collection suffered less and recovered more quickly than the green leaves. The degree of protection was even greater at low temperatures. When we illuminated the red and green leaves from their green undersurfaces, the anthocyanin layer could not protect the photosynthetic tissues, and both red and green showed the same degree of photoinhibition. Workers in other laboratories have evidence that anthocyanins also protect Antarctic mosses, pine needles and understory plants found in tropical rainforests.

Antioxidants by and for Plants

In addition to protecting plants from the usually short-term problem of photoinhibition, anthocyanins appear to protect plants from permanent damage by acting as antioxidants. One consequence of the absorption of intense sunlight in leaves is increased production of reactive oxygen species and free

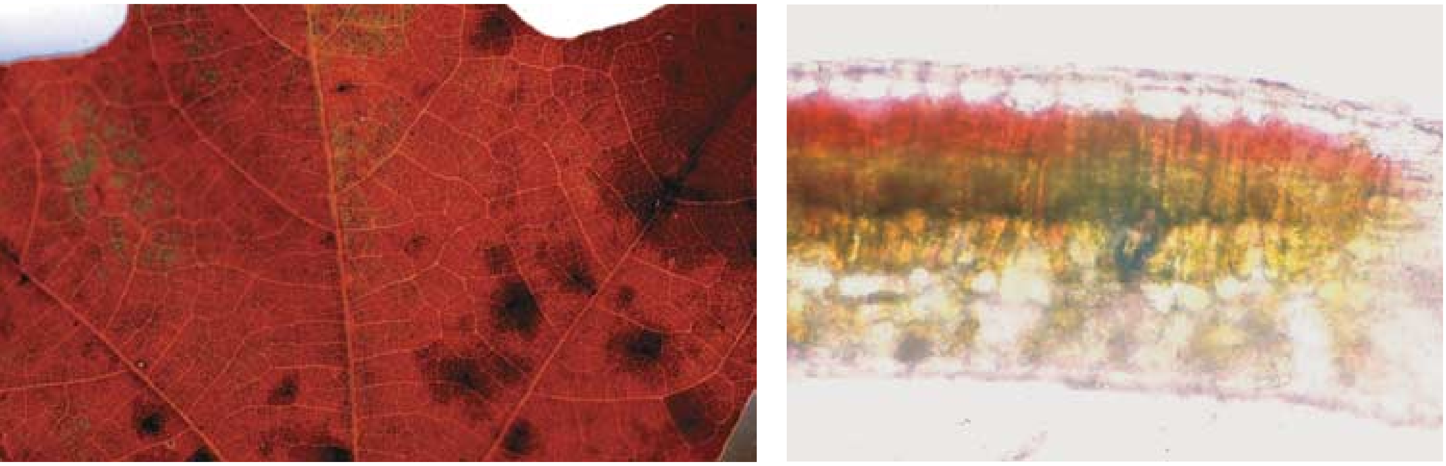

Figure 3. Many senescing leaves are red, as in the red oak (Quercus rubra, left). A transverse section (right) shows that the anthocyanin pigments responsible for this color are located in vacuoles of the long, thin palisade cells, which are stacked upright just under the upper epidermis.

radicals (molecules with unpaired electrons), such as singlet oxygen ($^1O_2$), superoxide ($\bullet O_2^-$) and the extremely toxic hydroxyl radical ($\bullet$OH). The presence of unpaired electrons makes most free radicals unstable and highly reactive. But not all of them are damaging to plants. Some are involved in the normal formation of lignins, which strengthen cell walls; others fight off pathogens. However, when the concentration of free radicals exceeds the ability of natural antioxidants to quench them—particularly during periods of stress—these reactive molecules can destroy the biological machinery around them, including membranes, proteins and DNA, potentially leading to cell death. Leaves, with their relatively high concentrations of oxygen from photosynthesis and with their frequent exposure to intense sunlight, seem particularly vulnerable to oxidative damage.

Anthocyanins have the potential to curtail photooxidative damage by absorbing green wavelengths of light, thus shielding chloroplasts beneath from a portion of the spectrum that they cannot exploit for energy production. Thomas Vogelmann, who recently moved from the University of Wyoming to the University of Vermont, and his colleagues have shown that the light energy that anthocyanins absorb does not subsequently transfer to the chloroplasts. Instead, the energy either is retained in the vacuole containing the anthocyanins (preliminary evidence suggests that energy makes the molecules vibrate and even hum) or, more likely, is dissipated gradually as heat. Consequently, the chloroplasts in red leaves produce fewer free radicals. Samuel Neill, a Ph.D. student at the University of Auckland, measured levels of superoxide produced by a suspension of chloroplasts taken from leaves of lettuce. When the chloroplasts were irradiated with white light, the amount of superoxide rapidly increased, whereas they produced much lower levels when irradiated with light that was of a similar intensity but had been passed through a green-absorbing filter. Clearly, the absorption of green light by anthocyanins impedes superoxide production.

Anthocyanins may also help a plant because they are potent antioxidants. Solutions of cyanidin, the most prevalent anthocyanin in leaves, have about four times more antioxidant capacity than ascorbic acid or vitamin E. People

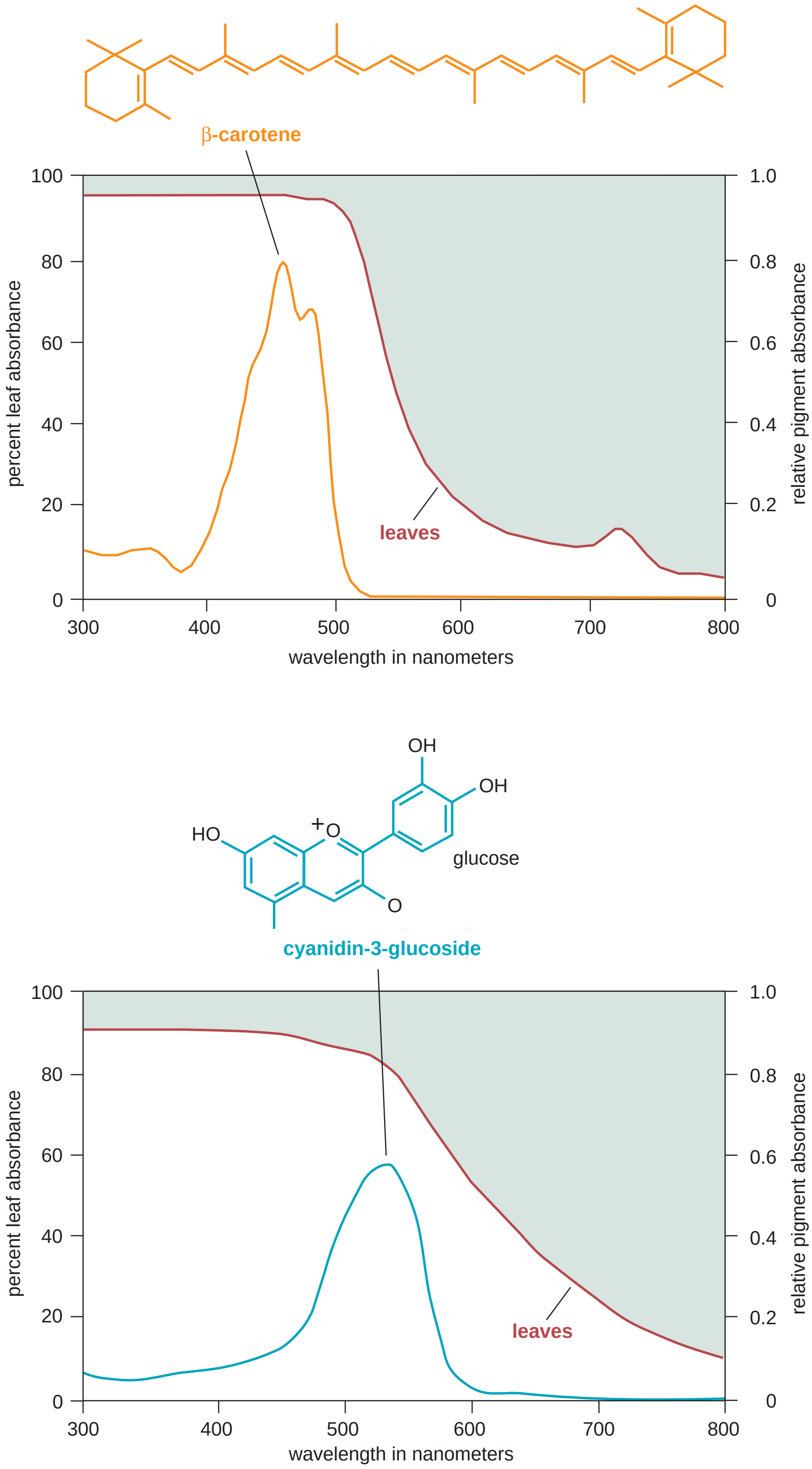

Figure 4. Beta-carotene molecules (top) add yellow color to senescing leaves. The light absorbance spectrum of leaves containing beta-carotene is compared with the absorbance spectrum of the molecule alone. The most common anthocyanin molecule in leaves is cyanidin-3-glucoside (bottom). Again, the absorbance spectrum of leaves containing the pigment is compared with the wavelengths of light absorbed by the isolated molecule. Because of the scattering of light within leaves, the absorbance spectra of the molecules are broader when they are in their native environment than when the pigments are isolated.

Figure 5. Red or purple color is often seen on the undersurfaces of leaves growing in the lower, more shaded parts of forests, especially in the tropics. The herb shown (Triolena hirsuta) grows in the tropical understory of Central American forests. Whether or not their undersides are red varies, making them a good subject for research. (Photograph courtesy of George Valcarce.)

who ingest anthocyanins from fresh fruit and red wine can improve the antioxidant status of their blood plasma. In 1999 James Joseph and many collaborators at the United States Department of Agriculture and at Tufts University demonstrated dramatic reversals in the decline of nervous system activity in mice that were fed anthocyanin-rich blueberries (Vaccinium corymbosum). Widespread reporting about these results led to a run on fresh blueberries in supermarkets across the country. Now a growing body of evidence shows the effects of anthocyanins as antioxidants, raising the possibility that perhaps these molecules are as health-promoting for the plants that produce them as for the animals that ingest them.

One of us (Gould) and his colleagues studied the potential for such protection in a New Zealand shrub, Pseudowintera colorata. Parts of the upper surfaces of its leaves are blotched red from the accumulation of anthocyanins. When we punctured the leaves with a fine needle, chloroplasts in the injured area produced strong bursts of the reactive oxygen compound hydrogen peroxide (H_2O_2), which we could detect and monitor over time. Chloroplasts in both the green and red areas produced hydrogen peroxide, but differences between the two regions became apparent within minutes of injury. Hydrogen peroxide continued to accumulate in the green portions for 10 minutes and then decreased only slowly. In contrast, levels in the red regions declined rapidly to background counts within the first five minutes.

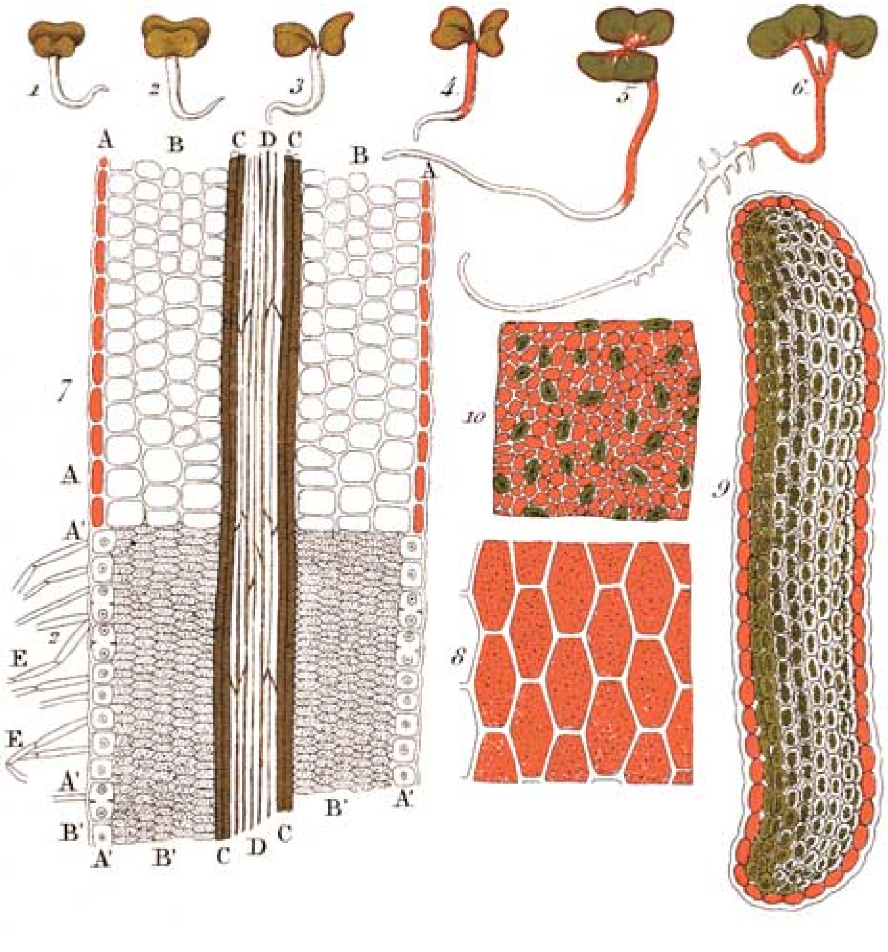

Figure 6. Investigators in the 19th century carefully observed the distribution of anthocyanins in different plants. Belgian botanist Édouard Morren published these microscope observations in 1858, showing the distribution of anthocyanins in the various organs of young seedlings of red cabbage (Brassica oleracea).

How exactly do the anthocyanins protect from oxidative damage? The phenomenon is rather enigmatic. Whereas the troublemaking oxygen molecules do their damage in the chloroplasts or in the cytoplasm, the anthocyanins are largely sequestered in cell vacuoles. Perhaps the anthocyanins, which are produced in the cytoplasm, carry out their antioxidative function there, before moving into vacuoles. Or maybe these organelles act as sponges for destructive hydrogen peroxide, which, unlike other reactive oxygen molecules, can migrate across the membrane of a vacuole.

Even if biologists figure out how it is that anthocyanins can protect plant cells from oxidative damage, it will be hard to say whether these pigments evolved primarily to scavenge free radicals or to combat photoinhibition. It is possible that these functions act simultaneously to protect plant tissues from intense sunlight. By absorbing light, anthocyanins would reduce the rate of both photoinhibition and photooxidative damage, and they could also neutralize whatever free radicals still ended up being produced. Both mechanisms could serve to protect plant tissues that are especially vulnerable to such damage, such as those under environmental stress or in developing leaves when the photosynthetic structures are being assembled.

Despite the protective functions of anthocyanins (particularly in bright, cold conditions), it is difficult to understand why plants invest energy to protect foliage that is about to fall off. Shielding the photosynthetic apparatus of a dying leaf would add little to the carbon supply of a tree and is unlikely to justify the metabolic cost of synthesizing an elaborate pigment

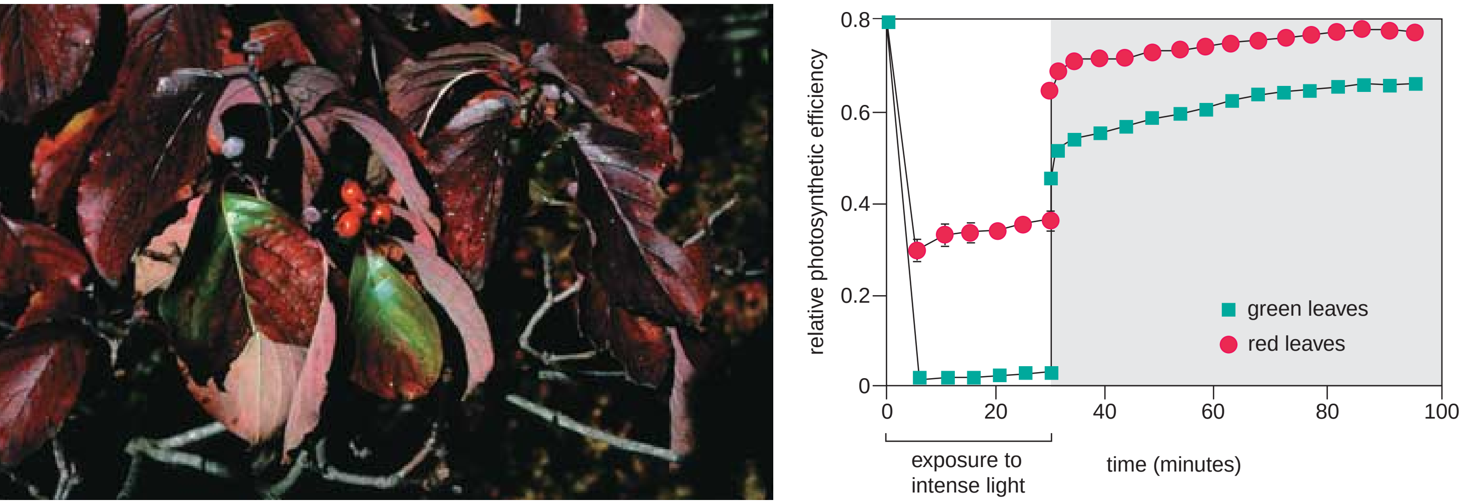

Figure 7. Red-osier dogwood (Cornus stolonifera), photographed in the Catskill Mountains of New York, has red leaves in the autumn as well as some persisting green leaves (left). Green and red leaves on the plant differ in their relative photosynthetic efficiencies (right). Red leaves lose less efficiency when exposed to intense light and recover more rapidly after the exposure.

molecule. But it is possible that the benefits continue into the next growing season, if, for example, the anthocyanins in autumn leaves act to allow the coordinated disassembly of the photosynthetic apparatus, particularly the breakdown of chlorophyll, before the leaves drop.

The critical issue may be the movement of nitrogen back into the plant. The photosynthetic apparatus in leaves contains much of the total nitrogen, which is wasted if the plant does not recover it before the leaves detach. Anthocyanins could protect this dismantling process in leaves and increase the amount of nitrogen shifted into the woody tissues of the parent tree. Although the explanation is simple enough, it may be difficult to obtain clear support for it. Anthocyanic concentration in foliage, which varies both among and within species, such as red maple (Acer rubrum), should be correlated with lower nitrogen levels in leaf tissues and higher efficiencies of absorption into the plant. But the efficiency of nitrogen absorption can vary a great deal, both among individual plants within the same species and in the same plant from year to year. It's possible that the increase in absorption may be rather small, and therefore hard to detect, and yet still provide significant advantage. We have obtained some preliminary evidence supporting this hypothesis from shrubs and trees at the Harvard Forest, but much more research will be required to properly test this prediction.

Ecological Functions of Anthocyanins

Some biologists have speculated that the red colors in leaves may protect them from being eaten. Anthocyanins are members of a class of plant compounds (polyphenols) that sometimes defend against predators, insects and microbes, but anthocyanins themselves don't seem to act as poisonous deterrents. However, the red appearance of leaves may warn animals that the leaves are unpalatable. In many tropical trees, for example, young red-purple leaves hang from branch tips until at the end of their development they rapidly become green. Phyllis Coley of the University of Utah has shown that these anthocyanic leaves have very low nitrogen content and are seldom damaged by herbivores. She believes that

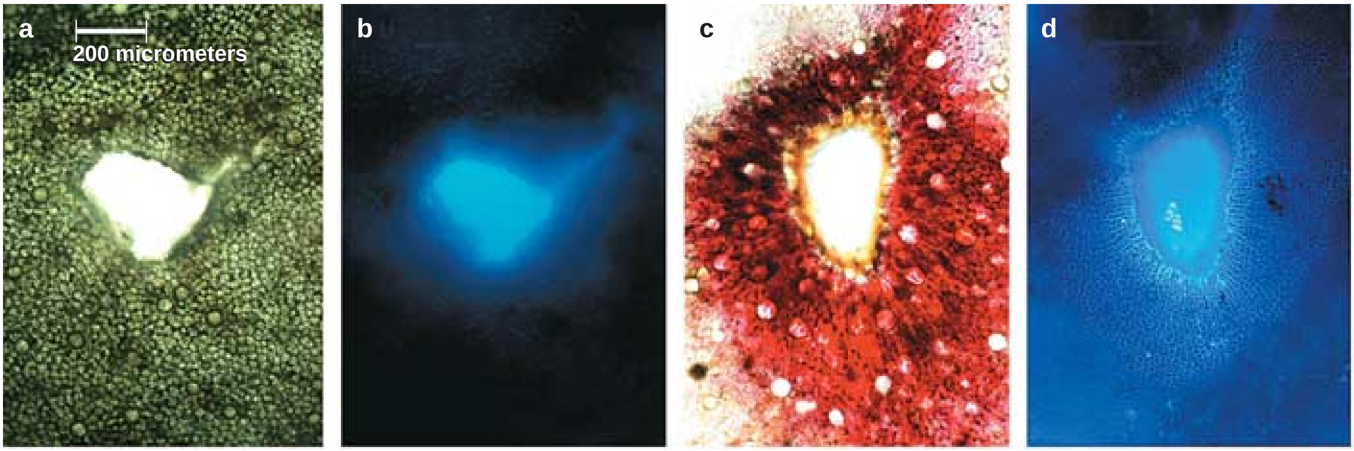

Figure 8. Puncturing the leaves of Pseudowintera colorata (the plant shown in Figure 1) with a needle leads to the release of hydrogen peroxide (H_2O_2), which can be revealed using a blue dye that loses its fluorescence when exposed to this highly reactive compound. When a green leaf blade was tested (a), it was flooded with hydrogen peroxide, as shown by the lack of fluorescence around the puncture (b), and levels decreased only slowly. But when a red portion of a leaf was pricked (c), hydrogen peroxide levels returned to normal within five minutes (d).

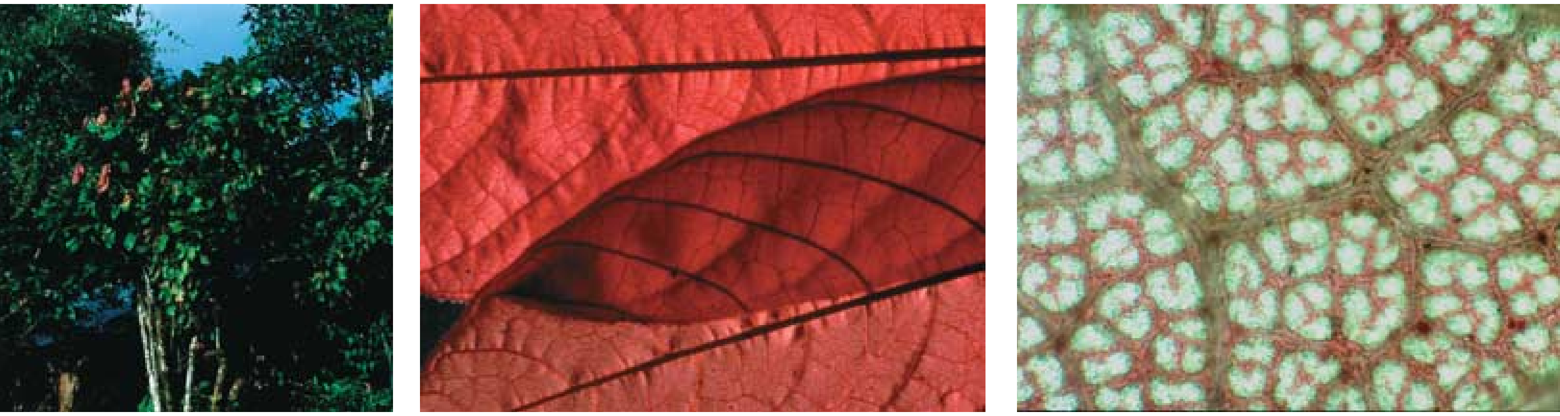

Figure 9. Anthocyanins are common in the developing leaves of tropical plants, such as the cocoa plant (Theobroma cacao). The shrub (left) and leaves (middle) were photographed in French Guiana. An even closer view through the leaf surface (right) reveals that anthocyanins are located exclusively in the tips of hairs and in cells surrounding leaf veins.

the anthocyanins may inhibit the growth of fungi that leaf-cutting ants cultivate and thus reduce leaf collection by the ants.

Paul Lucas of the University of Hong Kong and his colleagues have argued that young, red leaves in tropical forests are edible, and indeed important in the diets of some primates. They have shown that chimpanzees and monkeys of the Kibale Forest, in Uganda, prefer young, red leaves, which are highly palatable because of high protein levels and tenderness. Field tests of feeding activities suggested to them that red-to-green shifts in leaf color are important cues that led to the evolution of three-color vision in certain primates (including humans), unique among all mammals. In rare cases, anthocyanin color in leaves may attract animals to consume fruits and disperse seeds, but few of the red-senescing plants we have studied produce fruits that persist so late in the growing season.

It's conceivable that autumn coloration evolved to deter herbivores, but the deterrence would only lead to a selective advantage if it protected the trees from damage in subsequent seasons. The late William Hamilton of Oxford University and Samuel Brown, currently at the University of Montpellier II in France, argued that autumn coloration could warn aphids against laying eggs on trees defended with plant compounds. Thus, the autumn coloration could prevent leaves from being eaten the following year, when the eggs would have hatched. So far this research is based on literature surveys and modeling, with experiments and direct field observations yet to be completed.

Problems Remain

The research we have described in this article is a dramatic shift toward the solution of a long-standing mystery, but it is just a beginning. Many questions remain: For example, if anthocyanins fulfill an important physiological or ecological function for certain plants, why then do some plants not produce such pigmentation? And why aren't more leaves red all the time? Interestingly, one large group of flowering plants, in the order Caryophyllales, are

Figure 10. In a single order of flowering plants, red leaf color arises not from anthocyanins but from the presence of nitrogen-containing betalain pigments. Bougainvillea (Bougainvillea spectabilis) is a tropical, shrubby vine whose developing leaves and flower bracts are red because of betalains, specifically the compound betanin (top right). A section through a leaf shows that the pigment is located in the epidermis and hairs (lower right).

incapable of synthesizing anthocyanins, although they produce the precursor flavonoid pigments. Still, these plants produce red colors, in both vegetative and reproductive organs, but with a totally different class of pigments—the betalains. Beets are a good example. The nitrogen-containing betanin has similar absorbance characteristics to the anthocyanins and also is a strong free-radical scavenger. Do these molecules function as an equivalent to anthocyanins in this order of flowering plants? If so, how did they originate?

In some cases, genes that promote anthocyanin production in leaves may also promote production in flowers and other organs. This connection may confound efforts to interpret field trials that test whether plants with anthocyanins are more likely to survive than those without these pigments. But these problems are not insurmountable. For instance, investigators can compare the performance of normal plants with those of natural mutants—or genetically engineered ones—varying in the production of anthocyanin. We expect some exciting research on the functions of anthocyanins in vegetative organs in the near future.

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Links to Internet resources for further exploration of "Why Leaves Turn Red" are available on the American Scientist Web site:

http://www.americanscientist.org/articles/02articles/Lee.html