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# Why are higher plants green? Evolution of the higher plant photosynthetic pigment complement

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## ABSTRACT

**The physiological reason that higher plants are green is unknown. Other photosynthetic organisms utilize pigments that strongly absorb green light; therefore, there must have been natural forces that 'selected' the photosynthetic pigments found in higher plants. Based on previously published data and our recent findings about green light and photosynthesis within leaves (Sun *et al.*), a specific functional role is described for the primary photosynthetic pigments of higher plants, that were derived from green algal progenitors. The particular absorptive characteristics of chlorophylls *a* and *b* appear to perform two contradictory, but necessary functions in higher plants. Firstly, chlorophylls *a* and *b* absorb light for maximum utilization under non-saturating conditions, a function that is well understood. Secondly, they can act as protective pigments under over-saturating light conditions, when absorbed light is dissipated as heat. Under such conditions, a significant portion of light can also be efficiently utilized, especially in the bottom portion of the leaf, that is mainly illuminated by green light and not down-regulated. The second function may have been the selective force that gave rise to the extremely successful terrestrial plants, that evolved from green algae.**

*Key-words*: *Spinacia oleracea*; carbon fixation; evolution; green light; photosynthetic pigments.

*Abbreviations*: Chl, chlorophyll, NPQ, non-photochemical quenching; PAR, photosynthetically active radiation; PM, palisade mesophyll; PET, photosynthetic electron transport; SM, spongy mesophyll.

## INTRODUCTION

The physical attributes of the pigments involved in harvesting light were important contributing factors in the evolutionary selection of the chemicals used for photosynthesis (Franckel 1955; Evstigneev 1974; Blankenship & Hartman 1998). However, the reason that higher plants utilize the specific complement of chlorophylls *a* and *b* and carotenoids is a matter of speculation. The selective forces that drove the evolutionary selection are unknown, and we can ask the following questions: why don't higher plants also utilize pigments that absorb more strongly in the green region?; why are higher plants green?

The trivial answer to the latter question is physical (optical). Green algae and higher plants utilize chlorophylls *a* and *b* and a variety of carotenoids to capture light for photosynthesis (Evstigneev 1974; Glazer 1980). Other pigments utilized by photosynthetic organisms, such as chlorophyll (Chl) *c*, fucoxanthin, and phycobilins, absorb light in all regions of the visible spectrum (Haxo & Blinks 1950; Glazer 1980), but such pigments are not utilized by green algae and higher plants (Evstigneev 1974). Higher plant Chls and carotenoids most strongly absorb light in the red and blue regions of the visible spectrum. Green light is the least absorbed and the most reflected, so most leaves are green.

Many action spectra of dilute algal suspensions, that have a low Chl content, clearly show that green light is less effective than blue or red light at driving photosynthesis (Haxo & Blinks 1950; Shibata, Benson & Calvin 1954). The classic experiments of Englemann using aerobic bacteria, illustrate the same point (Englemann 1882). Consequently, it is a common misconception that green light is unimportant to photosynthesis (see most biology textbooks). In leaves that have a high Chl content, 80–90% of the green light impinging on the leaves is absorbed (Rabideau, French & Holt 1946; Moss & Loomis 1952; Inada 1976). Clearly green light is an important energy source that can be utilized by higher plants (Sun, Nishio & Vogelmann 1998 and citations therein).

The physiological reasons that plants with green algal progenitors were evolutionarily successful on land remain unknown and are probably more complex. Since plants evolved well before vision, there is probably no adaptive value in being 'green' with regard to co-evolution with animals; although vertebrate vision is most sensitive to green light. Insects, of course, have innumerable co-evolutionary relations with plants, many of which are based on floral colour, for example. Besides being important as a source of energy, light also controls many developmental aspects of plant growth. The presumption is made that the photosynthetic pigments for energy collection were selected prior to light-sensing systems for development. The evolution of light-sensing systems in plants is intriguing but will not be addressed in this article.

The role of green light in carbon fixation within leaves

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was recently demonstrated in my laboratory by Jindong Sun, who showed that green light drives carbon fixation deep within leaves (Sun et al. 1998). Under equivalent irradiance of broad-band monochromatic green or red light, $CO_2$ fixation rates for intact leaves on an areal or Chl basis were similar. However, on a Chl basis, carbon fixation under green light is substantially higher in the spongy mesophyll (SM) compared with fixation under either red or blue light (Fig. 1). These findings illustrate that green light is an important energy source for carbon fixation deep within leaves.

Both the palisade mesophyll (PM) and SM contribute significantly to carbon fixation across bifacial leaves (Mokronosov *et al*. 1973; Outlaw & Fisher 1975; Jeje & Zimmermann 1983; Nishio, Sun & Vogelmann 1993). However, the maximum carbon fixation across a spinach leaf occurs not at the top of the leaf, where light is maximal in spinach leaves (Terashima 1989; Cui, Vogelmann & Smith 1991), but in the middle of the PM (Nishio *et al*. 1993). The light absorption profile across spinach leaves is due mainly to Chl (this paper), whereas the pattern of fixation across the leaves is due to the distribution of Rubisco (Nishio *et al*. 1993) and light absorbed by the reaction centres. The reason for the disconnection of light at the top of the leaf with the carbon fixation profiles across the leaf is not understood.

Likewise, neither red, blue, nor green light gradients correlate with the carbon fixation profiles across leaves (Sun *et al*. 1998). Carbon fixation under blue light occurs mainly in the PM, whereas fixation under green light extends more deeply into the leaf. Fixation under red light occurs mainly at the top of the leaf, but extended more deeply into the leaf than under blue light, but not as deeply as under green light (Fig. 2). The sum of fixation under red, blue, and green light was equivalent to that under white light alone (Fig. 3). The difference at the bottom of the leaf suggests that 500 $\mu$mol $m^{-2}$ $s^{-1}$ of the monochromatic light was not saturating, as a similar difference in the bottom half of the leaf was shown with non-saturating white light (Nishio *et al*. 1993).

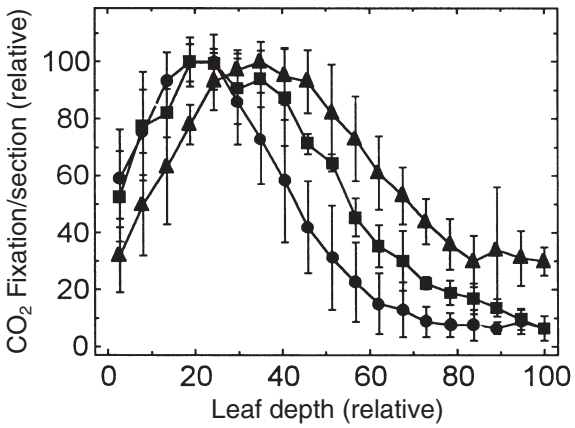


**Figure 2.** Relative carbon fixation across spinach leaves irradiated with 500 $\mu$mol $m^{-2}$ $s^{-1}$ of either blue (●), red (■), or green (▲) light (adapted from Sun *et al*. 1998).

The photosynthetic apparatus of higher plants must be highly adaptable to large changes in quantum flux (Ewart 1896, 1897; Björkman 1981; Ort & Baker 1988). Under full sunlight, the outer portion of the leaf canopy is oversaturated with light, whereas non-saturating conditions exist deep within the canopy. As a consequence, at the whole plant level, higher plant photosynthesis is generally limited by light because non-saturating light conditions occur even under full sunlight (Terrien, Truffaut & Carles 1957; Monteith 1965). Photosynthesis within the canopy is dependent on light that is transmitted through the leaves and/or on light flecks that may penetrate deeply into the leaf canopy (Monteith 1965; Fogg 1968; Pearcy 1990).

Photosynthesis in leaves of many $C_3$ plants saturates well below full sunlight (Franckel 1955; Terrien *et al*. 1957; Berry 1975), yet the leaves must be able to withstand full sunlight, and also be productive under conditions that are not light-saturating (Ewart 1897). Plants have evolved gross, physical approaches to deal with 'high' light, such as protective leaf and leaflet movement (Ewart 1897). Absorption of

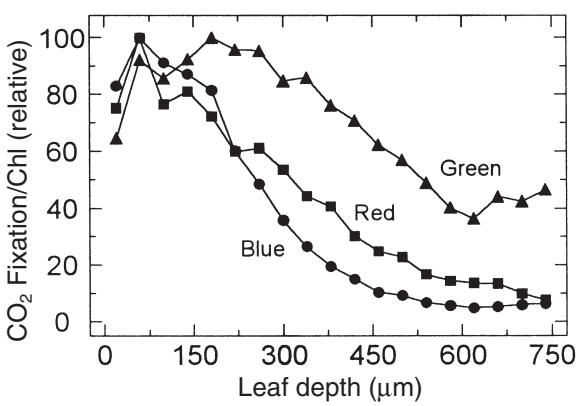


**Figure 1.** Relative carbon fixation/Chl under 500 $\mu$mol $m^{-2}$ $s^{-1}$ of either blue (●), red(■), or green (▲) light (Sun *et al*. 1998).

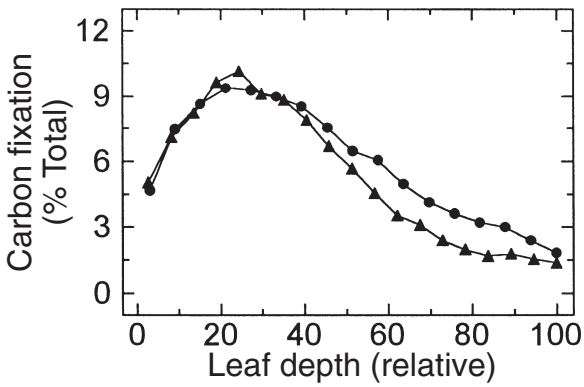


**Figure 3.** Sum of fixation under 500 $\mu$mol $m^{-2}$ $s^{-1}$ of green, blue, or red light (▲) compared to fixation under 800 $\mu$mol $m^{-2}$ $s^{-1}$ white light (PAR) (●).

light by non-photosynthetic pigments decreases photosynthetic efficiency. Conifers in particular exhibit decreased action in the blue region of the visible spectrum (Burns 1942). Carotenoids and flavonoids have a role in the decreased efficiency of blue light in driving photosynthesis (Gabrielsen 1948; Clark & Lister 1975; Inada 1976). Anthocyanins and other red pigments can also protect the photosynthetic apparatus from damaging light (Ewart 1897; Wheldale 1916), however, many higher plants do not have significant quantities of 'red dye' (anthocyanin) during the majority of the growing season (Ewart 1897). With regard to non-photochemical quenching (NPQ), carotenoids may be directly involved in dissipation of absorbed light energy by plants (Demmig-Adams & Adams 1992; Horton & Ruban 1994; Owens 1994; Niyogi, Björkman & Grossman 1997).

Thus, selective pressure for photosynthetic performance under high light conditions, that may cause photo-inhibition under stressful conditions (Ewart 1896, 1897, 1898) had to be moderated by equally strong pressures for light harvesting and photosynthesis under low light conditions, that often prevail. For example, a single leaf exhibits both 'sun' and 'shade' characteristics (Outlaw 1987; Terashima 1989; Nishio *et al*. 1993). Directional light on either the adaxial or abaxial leaf surface showed that photodamage to the PM or SM was dependent on the direction of illumination (Ewart 1897), but the upper portion of leaves is much more resistant to chronic photo-inhibition than the lower portion of the leaf (Sun, Nishio & Vogelmann 1996b).

In this paper, I present a hypothesis for why most terrestrial plants utilize the green algal photosynthetic pigment complement. The ramifications of some of our recent findings with regard to carbon fixation across leaves are discussed. I will address the importance of green light in the overall energy balance of photosynthesis of higher plants and discuss a role for green light, which may contribute more to photosynthesis than blue or red light under greater than saturating light conditions.

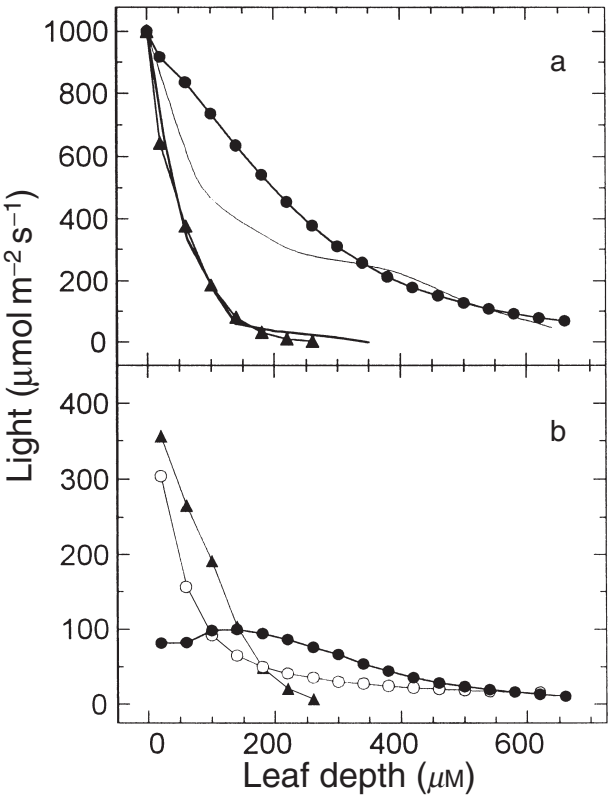


**Figure 4.** Measured and calculated light and absorbancy gradients across spinach leaves. (a) Light gradient. Measured light gradients (replotted from Cui *et al*. 1991) are shown as solid lines. The upper, thin line represents the measured green light gradient, and the thick, lower line represents the blue light gradient. The calculated blue (▲) and green (●) light gradients were calculated according to the text. (b) Light absorbancy. Light absorption was calculated based on a starting light value of 1000 $\mu$mol $m^{-2}$ $s^{-1}$. The blue light absorbancy curves based on the calculated light gradient or measured light gradient are similar. Shown is the curved based on the calculated gradient (▲). The green light absorbancy curves were significantly different depending on the light gradient used. Curve based on calculated gradient (●); based on measured gradient (Cui *et al*. 1991) (○).

## THE EFFECT OF CHL ON INTERNAL LIGHT MICROENVIRONMENTS WITHIN LEAVES

In spinach, the attenuation of light across the leaf is mainly due to Chl. The role of Chl on the 'average' light gradient across spinach leaves was estimated by simply assuming that Chl was homogeneously distributed in 80% acetone solutions of layered samples with 40 $\mu$m path lengths. The calculations were based on the extinction coefficients of Mackinney (1941) and the measured Chl *a* and Chl *b* within 40 $\mu$m paradermal leaf sections (Nishio *et al*. 1993). Figure 4(a) shows the calculated and measured (Cui *et al*. 1991) light gradient for 560 nm (green) and 450 nm (blue) light. The calculated and measured red (650 nm) light gradients are similar to the blue light gradient (not shown).

The calculated absorption profiles based on the measured and calculated light gradients are shown in Fig. 4(b). Absorption was derived by subtraction of measured light at one leaf depth from the measured light at a leaf depth above. The decrease in light would be due to absorption between the two leaf depths, although not all absorption would be due necessarily to Chl in the measured gradient. Measured and calculated gradients of absorption of red and blue light are highest at the top of the leaf and exhibit apparent extinction about midway through the leaf. In contrast, the green light absorption gradient extends through the leaf, as did fixation under green light irradiation (Sun *et al*. 1998).

The calculated green light gradient underestimates the attenuation of light at the top of the leaf but becomes identical to the measured gradient about midway through the leaf (Fig. 4a). The disparity in measured and calculated green light gradients at the top of the leaf is probably due to light scattering, which increases light absorption. Scattering effects are minimal where absorption is high, as in

the blue and red, so differences in the simple model acetone leaf and the real spinach leaf are minimal in the red and blue regions of the spectrum. The calculated red and blue light absorption curves nicely match the measured absorption curves (Fig. 4b). The differences in the model calculations and the measured light gradients for green light do not impact the interpretations presented in this paper.

Thus, it appears that Chl is one of the main physical components contributing to the light microenvironment within spinach leaves. Chlorophyll is sequestered in chloroplasts, that are distributed mainly around the borders of cells because of the central vacuole. Hence, Chl is not evenly distributed across a leaf (as assumed in the model calculation above). In addition, light reflects off cellular components, and the effective path length across a leaf is longer than the measured leaf thickness. The simple calculated light gradients based on a homogeneous distribution of Chl in 40 $\mu$m layers (Fig. 4a) remarkably matched the previously measured light gradients in spinach (Cui *et al*. 1991). These findings illustrate that Chl alone can account for a majority of the light absorption characteristics of spinach leaves, despite light scattering effects and the sieve effects due to the compartmentalized Chl. However, the apparent extinction of both red and blue light so close to the upper leaf surface is probably artifactual (for reasons just mentioned; also see Terashima 1989), since red and blue light drive photosynthesis deeper in the leaf than their respective light gradients suggest is possible.

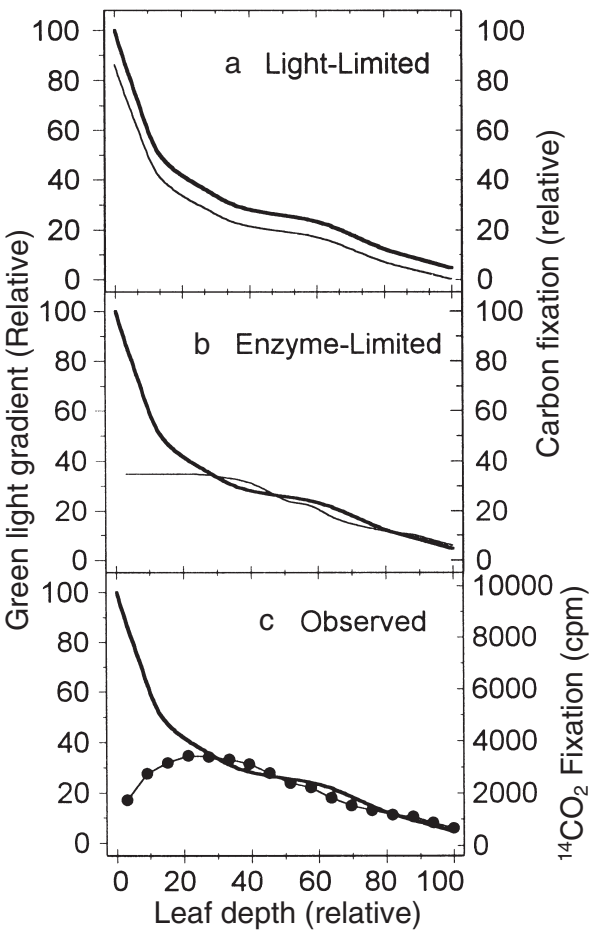


**Figure 5.** Relation between light gradient and carbon fixation across spinach leaves. The dark line represent the measured green light gradient across spinach leaves (Cui *et al*. 1991). The thinner line represent a model carbon fixation gradient. (a) Light-limited. Assumes that carbon fixation is light limited. The higher the light level, the greater the fixation rate. (b) Enzyme-limited. Assumes that carbon fixation is enzyme limited at the top of the leaf and that maximal enzyme exists in the upper part of the leaf. Once the light is saturating, the rate of carbon fixation does not increase. (c) Observed. The observed carbon fixation (●) is shown. The maximum fixation occurs mid-way through the palisade mesophyll.

Absorbed light decreased exponentially across the leaf (Fig. 4b). Photosynthetic electron transport (PET) is driven by absorbed light, so the capacity for PET does not necessarily correspond directly to the $^{14}CO_2$-fixation gradient across leaves. Carbon fixation gradients across leaves exhibit a maximum in the middle of the (PM), at a depth approximately 20–35% into the spinach leaves (Figs 3 and 5c).

Questions have been raised regarding the shape of the absorption profile across leaves, however. Calculated absorption profiles across *Catalpa* leaves (Richter & Fukshansky 1996) and spinach leaves (Evans 1995) based on our previously published spinach data (Nishio *et al*. 1993) show absorption profiles across leaves that generally match the shape of the fixation gradient we have measured (Nishio *et al*. 1993). Such calculated absorption curves, however, are in direct contrast to existing measurements of light within spinach leaves (Cui *et al*. 1991; redrawn in Fig. 4a; see also Terashima 1989), and our calculated gradients based on Chl distributions across leaves. Additionally, leaves can absorb significantly more light than can be utilized by the photosynthetic machinery (see Appendix). Resolving the discrepancy between the data (Cui *et al*. 1991; Terashima 1989) and the recent models will require additional measurements of total photosynthetically active radiation (PAR) across spinach leaves.

## MODELS OF LIGHT UTILIZATION AND OBSERVED LIGHT UTILIZATION WITHIN LEAVES

Two models of light utilization across leaves and the observed pattern are shown in Fig. 5. The 'light-limited' model (Fig. 5a) assumes that there is maximum substrate and enzyme for carbon fixation at the top of the leaf, where light is maximal, on average. Such a model has been presumed correct. The 'enzyme-limited' model (Fig. 5b) assumes that the top part of the leaf is light saturated, and that substrate and enzymes limit carbon fixation at the upper part of the leaf. Such a model would seem reasonable, because photosynthesis in leaves is saturated well below full sun light levels. Additionally resources for enzymes at the top of the leaf suitable for maximum sunlight light would be wasteful, since full sunlight is ephemeral.

Green light gradients measured in spinach (Cui *et al*. 1991) and carbon fixation gradients measured in spinach (Nishio *et al*. 1993) (Fig. 5c) cannot be adequately explained by either the light-limited or enzyme-limited model. Instead, there is a clear depression of carbon fixation at the top of the spinach leaf, that is related to the Rubisco concentration (Nishio *et al*. 1993). The red and blue light gradients are more disconnected from carbon fixation than the green light gradient shown.

While maximum absorption of light occurs in the upper part of the leaf, maximal carbon fixation does not occur there, because Rubisco is maximal midway through the PM (Figs 3 and 5c). The distribution of Rubisco and $CO_2$ fixation across spinach leaves strongly correlate (Nishio *et al*. 1993). Total polypeptide accumulation tends to occur at the same depth of the leaf, which is partly related to the amount of plant tissue (Sun, Nishio & Vogelmann 1996a). The maximum palisade cell surface area (hence $CO_2$ absorbing area) apparently occurs roughly where the Rubisco concentration peaks (Terashima & Hikosaka 1995). Deep within the leaf, however, there is a strong correlation between the gradients of green light, carbon fixation, and Rubisco (Fig. 5c). The amount of green light absorbed, based on the calculated or measured light gradient across leaves, is adequate to drive measured fixation rates in the leaf (calculations not shown).

Leaf optics has been recently reviewed (Vogelmann 1993). Leaves produce 'hot spots' due to epidermal focusing (Poulson & Vogelmann 1990; Vogelmann 1993; Vogelmann, Bornman & Yates 1996), and cells of the PM allow light to be transmitted more deeply into leaves than leaves without PM (Vogelmann & Martin 1993). Interestingly, the calculated green light absorption gradient (Fig. 4b), which did not account for light scattering, more closely corresponds to the carbon fixation gradient across spinach leaves than does the exponential absorption gradient of green light, based on the measured light gradients across spinach leaves (Cui *et al*. 1991) (Fig. 4b). However, as stated above, the calculations did not account for sieve effects and light scatter. Although the measured light gradients (Cui *et al*. 1991) represent an average light gradient, it is also possible that absorption, *in vivo,* in spinach leaves is somewhere between the simple calculated absorption gradient and the real absorption gradient calculated from Cui *et al*. (1991). It is noteworthy, that deep within the leaf where light piping and epidermal focusing effects are minimal and light scattering is maximal, the model green light absorption gradient and measured green light absorption gradients are similar. Thus, it is clear that green light is important in driving photosynthetic electron transport deep within leaves.

It is possible that a reductant and/or ATP shuttle may exist between the top of the leaf and the bottom of the leaf (Outlaw, Schmuck & Tolbert 1976), but the strong correlation between green light and carbon fixation suggests that such a shuttle is not necessary. *In vivo*, measurements of electron transport capacity across leaves will aid in the elucidation of the possibilities (Han, Vogelmann & Nishio 1999), and we continue to investigate this realm of possibilities. Nonetheless, the high level of light absorption compared with Rubisco and carbon fixation at the top of the leaf illustrates that NPQ and other photoprotective processes must be very high in the upper portion of the leaf, otherwise photodamage would be very large at the top of leaves (Sun *et al*. 1996b).

## ABSORBED LIGHT VERSUS INCIDENT LIGHT

In full sunlight, a mechanism for heat dissipation by most plants is required because generally speaking, many times more light impinges on leaves directly exposed to full sunlight than can be utilized. The Appendix illustrates one example, that there may be in excess of five to six times the light impinging on leaves than is required to drive photosynthesis. The calculations shown are based on incident light. If one assumes 85% absorption (Seybold & Weisweiler 1943; Rabideau *et al*. 1946; Moss & Loomis 1952; Gates *et al*. 1965), there is still four to five times more light absorbed than that required for the model rate of photosynthesis. Under stressful conditions, when the plant is not operating optimally, there would be more than four or five times the amount of energy needed to drive photosynthesis, since photosynthesis would be depressed. Please see introduction for discussion of the importance of light avoidance.

Given the extreme amount of light that impinges on leaves directly exposed to full sunlight, it is reasonable to think that the upper part of a leaf may act as a light filter to protect the underlying tissue (Starzecki 1962; Bolhar-Nordenkamph 1982; Nishio & Ting 1987). Starzecki (1962) went as far as to suggest that the PM is the minor photosynthesizing tissue in leaves, because he found that some plants do not have a developed PM. However, many plants are isobilateral with PM present in the top and bottom parts of the leaf, and they may have little or no SM (Clements 1905; Shields 1950, 1951; Fahn 1974). Some plants have PM only on the abaxial leaf surface (Fahn 1974). Isobilateral leaves are associated with xeromorphy, as the amount of SM decreases with increasing xerophytic conditions. Xeromorphy can be caused by nutrition, low water status, high light, an upright leaf position (allows more light on the 'bottom' compared to a horizontal leaf), or alpine conditions, for example (Clements 1905; Maximov 1929, 1931; Shields 1950). The idea that the light-exposed leaf surface affords protection to the underlying tissue remains plausible.

In 'window' plants, it has been suggested that the multiple epidermis, a water-storing tissue, might protect underlying photosynthetic tissue from high light because the multiple epidermis absorbs and/or reflects 30% of the incident light (Nishio *et al*. 1987), and changes in epidermal transparency have been suggested as a possible mechanism of protection from high light (Ewart 1897). It is also likely that protective mechanisms exist in the upper portion of a leaf that is normally exposed to light (Ewart 1897; Powles & Björkman 1982; Sun *et al*. 1996b).

Plants must be able to cope with a wide range of light conditions on a daily basis. During cloudy days and at dusk or dawn, leaves in the outer canopy may receive less than saturating light. Leaves within the canopy, even on clear days, may be light-limited during the majority of the photoperiod. On the other hand, full sunlight provides significantly more energy than can be utilized by the photosynthetic electron transport system of most $C_3$ leaves, so energy dissipative mechanisms are important (Demmig-Adams & Adams 1992), and such dissipative mechanisms are more prevalent at the light-exposed top of the leaf. Hence, under greater than saturating light, the percentage of absorbed green light utilized for photosynthesis must be higher for green light than for blue or red light, since more blue and red light are absorbed at the top of the leaf.

## VERTEBRATE VISION VERSUS HIGHER PLANT LIGHT ABSORPTION

From an evolutionary perspective, it is of interest to compare vision in land animals to the light absorptive characteristics of pigments in higher terrestrial plants. In many cases higher plant progenitors exhibit complementary chromatic adaptation; that is, maximum absorbancy in the region of the spectrum that provides the most energy at the particular habitat in which the organism is growing (e.g. Haxo & Blinks 1950; Glazer 1989). Chromatic adaptation to maximize absorption of ambient light is often found where light is limiting.

Green light provides the greatest amount of radiant energy that reaches the earth's surface (see Kirk 1994). Like many higher plant progenitors, humans and other animals have evolved a keen sensitivity to green light relative to blue and red (Wald 1968; Bridges 1970; Kropf 1977). Human green and 'red' cone cell types have pigments that strongly absorb in the green region of the spectrum. The other cone cell type absorbs mainly in the blue. As a result, in bright light, human vision has its greatest sensitivity in the green (Fig. 6) (Wald 1968); (see also Bridges 1970; Fein & Szuts 1982), although there is excellent sensitivity across the visible spectrum. Under low light conditions visual sensitivity in many animals is shifted even more towards green light, with a $\lambda_{MAX}$ centred around 500 nm.

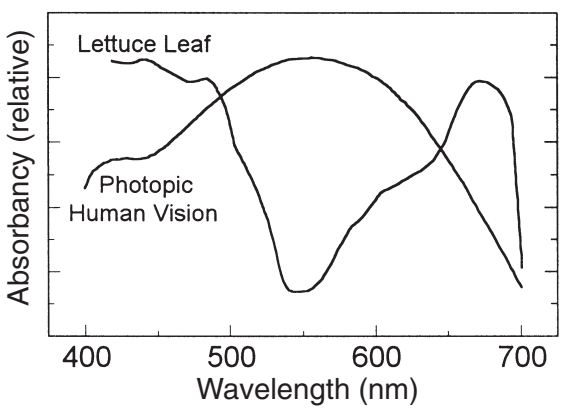


**Figure 6.** Relative higher plant leaf absorbancy in relation to human photopic vision. Absorbancy spectrum of a leaf from lettuce was redrawn from (Rabideau *et al*. 1946) and the absorbancy spectrum of the three cone cell types involved in human photopic vision redrawn from (Wald 1968). The spectra are relative, and illustrate that there is a clear difference in the maximum wavelength of absorption between the pigments used by higher plants and animals. Please note that a greener leaf would have a higher relative absorbancy in the green region, but the red and blue absorbancy would change relatively little.

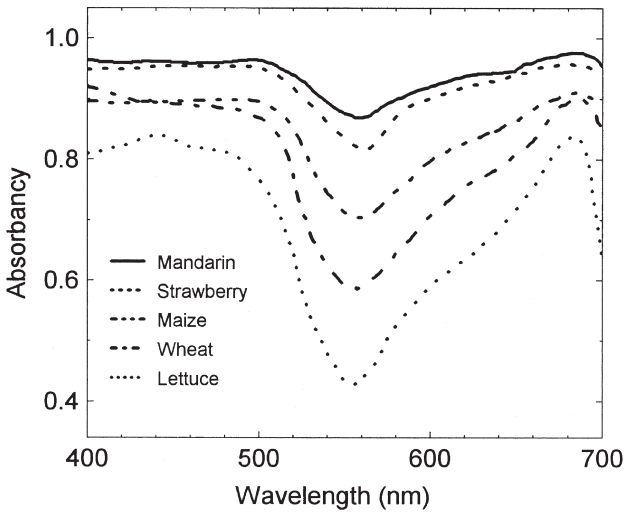


**Figure 7.** Absorption spectra of leaves from five different species. Figure adapted from Inada (1976).

In contrast, Chl's *a* and *b* and carotenoids combined have their lowest extinction in the green region of the electromagnetic spectrum (Fig. 6). In essence, higher plants have a green 'window', as illustrated in Fig. 7 (redrawn from Inada 1976). Leaves of satsuma mandarins (*Citrus unshiu* Mark., Okitsuwase), that are dark green absorbed most of the blue (96%), green (87%), and red light (97%) impinging on them. On the other hand, lettuce (*Lactuca sativa* L., Great Lakes no. 366), which had less Chl, absorbed only 43% of the green light, but still absorbed more than 80% of the light across the blue region of the spectrum. In other words, doubling the absorbancy in the green, only increased the absorbancy in the blue region of the spectrum by 15%. The absorption is not quite as strong across the red region, but the maximum absorbancy is also greater than 80%. Thus, even in leaves with relatively low Chl concentrations, the blue light is still mainly absorbed, and much of the red light, as well. It is worth noting that the red pigment, anthocyanin, often thought to protect photosynthesis from high light, is reasonably complementary to the green light window.

Of the light effectively absorbed by higher plants, blue light has the most energy per photon, yet it has the lowest action, due in part to 'screening' by carotenoids. The action spectrum of photo-inhibition shows that blue light inhibits

photosynthetic electron transport (oxygen evolution) (Ewart 1898; Jones & Kok 1966) more than equivalent fluxes of red or green light. Presumably there is a reason that higher plants maintain a complement of photosynthetic pigments that exhibit the least amount of absorbancy in the green region of the spectrum, which again, is the region of the spectrum that provides the greatest amount of energy on our planet's surface (Kirk 1994).

## WHY ARE HIGHER PLANTS GREEN?

Why would terrestrial animals evolve to be sensitive to the greatest radiant energy source, and terrestrial higher plants evolve a pigment system that absorbs the least in the same spectral region? Certainly, there must have been selective pressures for the disparate evolutionary paths. For animals, which utilize retinal in combination with different opsin proteins (Wald 1968), natural selection for sensitivity to the ambient light environment is a sound and reasonable argument (Fein & Szuts 1982). Note that the composition of the animal lens greatly impacts the absorbancy, because it can filter out UV light, even though the photoreceptors are sensitive to UV. Similarly plants possess flavonoids, that can act as a UV filter, in the epidermal cells.

For higher plants, the answer is not as obvious. The pigment complement utilized by higher plant green leaves may have been selected for protection against photoinhibition (see reference to Pringsheim in Ewart 1898 (p. 395), as well as for efficient light harvesting to drive photosynthesis. Besides the physical methods for avoiding high light, such as leaf movement and red dyes, plants also possess biochemical methods for high light tolerance (Ewart 1897; Franckel 1955). Excess light energy absorbed by Chl and carotenoids at the top of the leaf could be channelled to non-destructive decay pathways when NPQ is operating (Demmig-Adams & Adams 1992). Carotenoids contribute directly to the inefficiency of blue light under high light conditions, which is considered photoprotective. Under low light, however, when NPQ is not induced, all the energy absorbed by Chl could be used for electron transport.

When leaves are exposed to greater than saturating light, the excess light energy absorbed at the top of the leaf must be dissipated as heat. Heat dissipation at the leaf surface is feasible, and evapotranspiration is a major component of such dissipation of energy. Any number of possible heat dissipation mechanisms may be involved (Demmig-Adams & Adams 1992; Sun *et al*. 1996b). Indirectly, non-plastid absorption by cellular components decreases photosynthetic action (Strain 1950; Inada 1976); and xerophytes tend to have PM that are decreased in diameter, thereby increasing their cell wall per plastid (Shields 1950). The increase in cell wall may afford protection, as cell walls, while being somewhat transparent, also absorb light (see Strain 1950, 1951); they also may aid in transmission of light more deeply into the leaf. Future research aimed at understanding the specific mechanisms that control energy dissipation across the leaf will be enlightening.

The presently evolved absorption characteristics of higher plant Chl's *a* and *b* allow optimal photosynthesis under saturating and non-saturating light conditions. Under high photon flux, the blue and red light are efficiently absorbed in the upper part of the leaf. Since NPQ is linked to light absorbed by Chl and carotenoids, blue and red light absorbed at the top of the leaf must contribute mainly to such quenching when it is induced. Green light absorbed at the top of the leaf will also be proportionately dissipated. Thus, light absorbed by Chl and carotenoids at the top of the leaf protects the lower region of the leaf from high photon flux. In particular, the blue light, will be 'screened' out and its energy will be dissipated as heat (see Fig. 7).

In contrast, deep within the leaf where light fluxes are decreased, and there is a strong correlation between the green light gradient and carbon fixation (Fig. 5c), NPQ will be disengaged; and green light will efficiently drive photosynthesis (Sun *et al*. 1998). Under low light, however, maximum absorption of blue and red light, when NPQ is not active, will ensure efficient photosynthesis under non-saturating light conditions (in both the upper and lower region of the leaf).

It appears that the particular complement of photosynthetic pigments in higher plants evolved to maximally utilize green light (Sun *et al*. 1998). Instead of having a maximum extinction in green light, however, higher plant photosynthetic pigments exhibit the lowest extinction in the green. Hence, modulation of green light absorption by leaves and the leaf canopy can occur by varying leaf thickness and the Chl content in leaves, whereas red and blue light absorption varies relatively little (e.g. Rabideau *et al*. 1946; Strain 1951; Moss & Loomis 1952; Inada 1976; see Fig. 7).

In conclusion, the particular complement of photosynthetic pigments used by higher plants is well suited to the highly variable light environment on land. Under non-saturating light, maximal utilization of light is possible, because NPQ is not induced. Under saturating light conditions, when NPQ is engaged, high quantities of blue and red light energy absorbed in the upper, light-exposed portion of the leaf can be dissipated as heat. Green light transmitted deeply into the leaf, however, can effectively drive photosynthetic electron transport, where NPQ will not be engaged. If other pigments such as fucoxanthin, biliproteins, or Chl *c* were utilized by higher plants, the green light window would be effectively closed, and such dynamic absorption and utilization of light would not be possible.

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## APPENDIX

Two example calculations that illustrate the excess amount of light that impinges on leaves in full sunlight are shown. The calculation does not account for non-photosynthetically active light energy, which is equivalent to about half the light energy impinging on leaves.

### Example 1

A reasonably conservative calculation shows that if a plant is fixing carbon at a rate of 20 $\mu$mol $CO_2$ $m^{-2}$ $s^{-1}$ * in full sun or 2000 $\mu$mol quanta $m^{-2}$ $s^{-1}$, at an operating efficiency of about 20 quanta/$CO_2$ fixed (which generally accounts for photorespiration and other reductive processes in a healthy plant), then there is five times more light impinging on the plant than can be utilized (Eqn 1).

$$\frac{2000\ \mu\text{mol quanta}}{\text{m}^2 \cdot \text{s}} \times \frac{\text{m}^2 \cdot \text{s}}{20\ \mu\text{mol CO}_2} \times \frac{\text{CO}_2}{20\ \text{quanta}} = 5. \qquad (1)$$

*Plants with rates of photosynthesis of 80 to 100 $\mu$mol $CO_2$ $m^{-2}$ $s^{-1}$ are known, but rare. Even sunflower and soybean can have rates near 70 $\mu$mol $CO_2$ $m^{-2}$ $s^{-1}$; although under most agricultural conditions, such rates would not be realized during midday. The rate of 20 $\mu$mol $CO_2$ $m^{-2}$ $s^{-1}$ is representative of a reasonably fast rate of photosynthesis for any number of plants (Robert Pearcy, personal communication). Many plants do not reach the carbon fixation rate shown.

### Example 2

Assume a leaf contains 500 $\mu$mol Chl $m^{-2}$, then the amount of light that could be absorbed by a single Chl molecule is 4 quanta $s^{-1}$ (Eqn 2).

$$\frac{2000\ \mu\text{mol quanta}}{\text{m}^2 \cdot \text{s}} \times \frac{\text{m}^2}{500\ \mu\text{mol Chl}} \times \frac{4\ \text{quanta}}{\text{Chl} \cdot \text{s}}. \qquad (2)$$

Four quanta $s^{-1}$ $Chl^{-1}$ is equivalent to 250 ms $quanta^{-1}$, which is not fast enough for measured electron transport rates of 200 electrons $s^{-1}$. However, only 1 in 300 Chl's is a reaction center (RC), so

$$\frac{4\ \text{photons}}{5 \cdot \text{Chl}} \times \frac{300\ \text{Chl}}{\text{RC}} = \frac{1200\ \text{photons}}{\text{RC} \cdot \text{s}}. \qquad (3)$$

Since maximal measured photosynthetic electron transport is 200 electrons $RC^{-1}$ $s^{-1}$ (5 ms turnover due to limitation of plastoquinol reduction), then

$$\frac{1200\ \text{photons}}{\text{RC} \cdot \text{s}} \times \frac{\text{RC} \cdot \text{s}}{200\ \text{electrons}} = \frac{6\ \text{quanta}}{\text{electron}}. \qquad (4)$$