

SPECIAL ISSUE PAPER

Green light: a signal to slow down or stop

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Abstract

Light has a profound effect on plant growth and development. Red and blue light best drive photosynthetic metabolism, so it is no surprise that these light qualities are particularly efficient in advancing the developmental characteristics associated with autotrophic growth habits. Photosynthetically inefficient light qualities also impart important environmental information to a developing plant. For example, far-red light reverses the effect of phytochromes, leading to changes in gene expression, plant architecture, and reproductive responses. Recent evidence shows that green light also has discrete effects on plant biology, and the mechanisms that sense this light quality are now being elucidated. Green light has been shown to affect plant processes via cryptochrome-dependent and cryptochrome-independent means. Generally, the effects of green light oppose those directed by red and blue wavebands. This review examines the literature where green light has been implicated in physiological or developmental outcomes, many not easily attributable to known sensory systems. Here roles of green light in the regulation of vegetative development, photoperiodic flowering, stomatal opening, stem growth modulation, chloroplast gene expression and plant stature are discussed, drawing from data gathered over the last 50 years of plant photobiological research. Together these reports support a conclusion that green light sensory systems adjust development and growth in orchestration with red and blue sensors.

Key words: Photomorphogenesis, phytochrome, plant development.

Introduction

In 1990, a ten-year-old student discussed his science fair project with an evaluating judge. Three clear two litre soda bottles had been hand-coloured with translucent permanent marker. The layers of scribbling were so thick that they almost completely obscured the experimental subject, a solitary bean seedling growing in moist soil, negotiating an avenue of early photomorphogenic growth. The student's hypothesis was that different colours of light would have varying effects on plant growth. His results (and over five decades of photomorphogenic research) supported his hypothesis. The plants grown in red and blue bottles possessed expanded green leaves and compact stems, while those in green ambient environments maintained characteristics reminiscent of dark-grown seedlings. His interpretation was that since plants are green, they reflect green light, therefore green light is not useful for plant growth. His corollary was that blue and red light must be important for photosynthesis and that is why the plants grew better under those conditions. The impressed judge was a graduate student in plant biology from a nearby university and recognized the student's sound interpretations while handing him a first-place ribbon.

Almost two decades later the same judge (perhaps an author of this work) might ask that student to expound further on his initial interpretations. Perhaps a more complete conclusion is not just that red and blue promote plant development, but also, that green wavebands work against it. Biology has a habit of employing opposing systems to tightly monitor, adjust, and constrain developmental programmes, and a negative influence of a green light sensory system would be intuitively consistent with most models. Inspection of the classical and contemporary literature surrounding light-mediated plant developmental research presents several reports that support this view. For the purposes of this review 'green light' is expanded to represent the green and yellow portions of the spectrum

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(500–600 nm). Although green wavebands have been traditionally considered to be developmentally inconsequential outside of their partial forward stimulation of red and blue light responses, the literature is punctuated with cases where monochromatic or broadband green light treatment elicits effects on plant growth and development that do not conveniently dovetail with described light sensory paradigms. Analysis of this literature base unveils a common theme, that green wavebands tend to temper, if not negate, the effects of blue and red wavebands.

Since plants need light, why would light energy antagonize plant development? One need not search farther than the far-red portion of the electromagnetic spectrum to answer that question. Simple yet elegant experiments by Borthwick *et al.* (1952) illustrated that these far-red wavebands, inefficient for photosynthesis, impart potent environmental information. Generally, far-red light counters the developmental processes initiated by red light, and the ratio of red to far-red dictates the activity of molecular, biochemical and morphological processes (Quail, 2002; Devlin *et al.*, 2003; Chen *et al.*, 2004; Casal and Yanovsky, 2005). This is a prime example of how a light quality almost useless to plant metabolism potentially adjusts plant form, composition, and adaptive strategy to optimize light capture when quantities and/or qualities are unfavourable. Could green light convey similar information, at least under certain conditions?

Analysis of work throughout the 20th century suggests that it is likely. In the absence of cloned, sequenced, and characterized light sensors, scientists carefully described physiological and developmental responses to discrete portions of the light spectrum and its invisible fringes. Many seemingly green-specific responses were observed. Retrospective analysis places a subset of these phenomenologies into the camps of phytochrome and cryptochrome response. Some data are difficult to interpret, as experiments were usually performed under broadband conditions not exclusively emitting green light. Early experiments measured light in foot-candles or ergs, did not equalize light treatments across the spectrum, and/or used combinations of light treatments that obscure modern interpretation. However, occasional tests indicate, often in no unclear manner, that green wavebands conjure a specific suite of actions not intuitively attributable to red, far-red, or blue light and their cognate receptors. Later discoveries in *Arabidopsis thaliana* defined the genetic elements that transduce red, blue, and far-red signals. The characterization of photosensory mutants now provides tools that allow green responses to be studied in isolation from other light sensory inputs. Many of the responses induced from the green portion of the spectrum are counterintuitive, often opposing normal light effects (Klein *et al.*, 1965; Ahmad *et al.*, 1998; Frechilla *et al.*, 2000; Talbott *et al.*, 2002; Eisinger *et al.*, 2003; Folta, 2004; Dhingra *et al.*, 2006; Bouly *et al.*, 2007). Others are

completely irreconcilable yet interesting observations that merit additional investigation with a more comprehensive set of controls (Sommer and Franke, 2006).

The relevance of green light responses is predicated on the assumption that there are contexts where a plant may encounter enriched green conditions. Such states abound in the natural environment. Under the cover of leaves plants experience a pronounced contrast from unfiltered solar illumination. Primarily, there is a decrease in radiant flux and a shift in the ratio of visible wavelengths to far-red light (Fig. 1). The understory of a canopy is rich in far-red light as blue and red light have been removed by overhanging foliage. This depletion shifts the ratio of blue and/or red light to green light, as green light is readily reflected from and transmitted through plant tissues. Green light is efficiently transmitted through the plant body, playing more of a role in photosynthesis than red or blue in some contexts (Sun *et al.*, 1998), suggesting green light may prove useful as a signal to tissues not directly exposed to the light environment. Potential green light effects may also vary with developmental context. An etiolated seedling emerging through the soil has negligible chlorophyll, and green light is just as penetrant as blue, red, and far-red light. It is naïve to think that nature would not identify and capitalize on the information present in these prevalent environmental conditions.

Phytochromes and cryptochromes are green light receptors

It is difficult to characterize pure green light responses because phytochromes and cryptochromes are green light receptors. The phytochromes are principally thought of as red/far-red reversible pigments, yet they absorb well in the blue portion of the spectrum. These receptors are

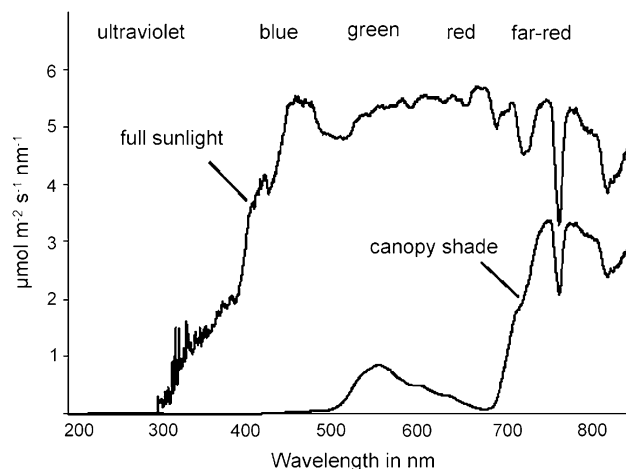


Fig. 1. Quantum energy distribution of full sunlight and under the shade of leaves. Light conditions were measured at noon in mid-April in Gainesville, FL (29.67° N), using a StellarNet spectroradiometer.

extremely sensitive to all light qualities, especially in dark-grown seedlings where light labile phyA is abundant (Goto *et al.*, 1993) and will initiate responses to minor illumination from light qualities across the spectrum. In *Arabidopsis*, green light stimulates germination effectively through phyA and phyB (Shinomura *et al.*, 1996). Green light establishes an active phy pool and even the most miniscule 'safelight' green light treatments activate robust plant responses (Mandoli and Briggs, 1981; Steinitz *et al.*, 1985; Dhingra *et al.*, 2006).

The cryptochromes regulate plant responses to blue and UV-A light (Lin, 2002; Spalding and Folta, 2005), and recent evidence indicates biological activity of a long predicted green-sensing state (Banerjee *et al.*, 2007; Bouly *et al.*, 2007). Malhotra *et al.* (1995) described the chromophore content of these photolyase-like photoreceptors, unveiling that the chromoproteins contained a flavin and a pterin as the light-excitabile moieties. The presence of both the flavin and the pterin suggested that cryptochromes relied on intramolecular electron transfer as part of their signalling mechanism (Malhotra *et al.*, 1995). Lin *et al.* (1995a) overexpressed *Arabidopsis Cry1* in transgenic tobacco. The overexpressors exhibited hypersensitivity not only to blue, but also to broadband green light, indicating that cryptochromes could direct stem growth inhibition even when stimulatory wavelengths are red shifted. However, recent reports by Bouly *et al.* (2007) and Banerjee *et al.* (2007) show that the green absorbing state of cry1 and cry2 reverses blue light-induced responses. These findings are discussed in detail in the final sections of this review. In addition, it appears that at least one additional light sensor system, or a new twist or unfound redundancy within those already identified, mediates specific effects to green wavebands, and these too tend to arrest or attenuate the physiological hallmarks associated with normal photomorphogenic progression. Therefore, green light responses can be characterized as those that are *cryptochrome dependent* and those that are *cryptochrome independent*.

Clearly, plants have sensitive green light sensors, the phytochromes and cryptochromes, but their efficiency in processing the green light signal is poor relative to their ability to respond to red and blue wavebands. With this in mind green light effects could be the result of low-level coaction between red and blue sensory systems, as outputs from minimal phy and cry activation may present what mistakenly appear to be green specific phenotypes. This interpretation likely deprioritized study of green light effects. However, today researchers possess genetic and physiological tools that allow green light effects to be teased apart from those induced by developmentally dominant wavelengths. Today studies of green light effects are made possible by the availability of high power narrow bandwidth light sources, access to double/triple photoreceptor mutants, and growth assays with great

temporal sensitivity. Together these tools have been used to characterize the often subtle effects of green illumination in highly plastic developing seedlings and in light-sensitive developmental transitions in mature plants.

Early green light effects on vegetative growth

The book '*Experimental control of plant growth*' by Frits Went (1957) describes an experiment that tested the effects of spectral quality on tomato (*Lycopersicon esculentum*) seedling dry weight. Plants were grown under various light qualities and different fluence rates for 6 d. Light was provided by fluorescent bulbs, and specific portions of the spectrum were excluded using gelatin theatre filters. A subset of the data has been reproduced in Fig. 2. The data indicate that seedlings grown under low fluence rate red and blue light possess more vegetative tissue than those grown under the same fluence rate of white light (principally red, blue, and green light). This result is expected because blue and red wavebands are efficient in promoting development and driving photosynthesis. White light contains a green component, so at any given fluence rate the added green is present at the expense of red and blue. However, as fluence rates increased an interesting trend emerged. Plants grown under a complete spectrum gained a finite mass before reaching a plateau. No matter how much the fluence rate increased, the mass stayed the same, indicating that the potential for fluence-rate-dependent vegetative growth was saturated. However, seedlings grown under the lavender filters (where the green component was significantly

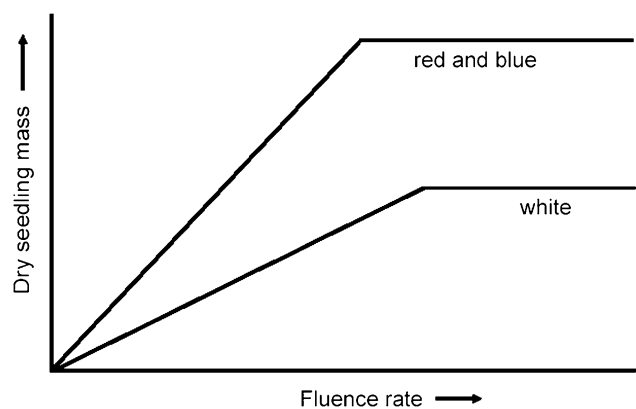


Fig. 2. Data reproduced from Went's green-depletion experiments in 1957. Tomato seedlings were grown under white light or lavender filters (lower green) and the dry mass of seedlings was measured after 6 d. The data show that, at low fluence rates, red and blue light are more efficient than white light (red, blue, green) in influencing vegetative growth, as expected. However, at higher fluence rates the white light-grown plants achieve a lower biomass than those grown in green depleted conditions, even when light intensity is high. The author interpreted these findings as evidence of inhibition of growth by green light. (Figure adapted from F Went, *Experimental Control of Plant Growth*, 1957).

reduced) achieved higher dry weight at saturation than seedlings grown under white light. From these data Went concluded that there was an 'inhibitory effect of green light' (Went, 1957). Today these results may possibly be reconciled as the negative cry-dependent or cry-independent effects on seedling development and growth.

Green light affects organ growth and stature

In the 1960s Klein and colleagues reported that conspicuous phenotypes could be observed when plants were grown in environments with depleted or supplemental green light. Early plant tissue culture techniques involved growth of tissues in darkness because light repressed culture growth. The most inhibitory light qualities radiated from fluorescent bulbs. Klein tested the action spectrum of growth inhibition and found that the most deleterious light quality was green light, peaking at 550 nm (Klein, 1964). This study was extended to mature plants. Rapidly growing marigold (*Tagetes erecta* L.), tomato, and impatiens (*Impatiens balsamina* L.) plants were grown under lavender filters using fluorescent and incandescent bulbs (Klein *et al.*, 1965). Here the green component was reduced significantly, shifting the ratio of green versus blue or red. Additional trials added supplemental green light to the white light treatments. Specifically, when grown under green-depleted conditions marigold height, fresh weight, and dry weight increased 30–50% over full-spectrum treatment, consistent with the results from Went (1957). The authors concluded that removal of green light enhanced plant growth. However, contemporary critical assessment of the data tempers this interpretation, as what was scored as 'enhanced growth' may simply be a lower amount of PAR, as the lavender filter significantly reduced blue light as well as green. This would also explain the taller plants, as they exhibited less growth inhibition. The white light sources emitted 1000 and 500 footcandles (approximately 200 and 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively), yet the intensity with filters was not provided, so these results are difficult to interpret. The more informative experiments were those that tested the consequence of added green light to full spectrum. Here plants were shorter and had less fresh/dry mass. Surprisingly, higher PAR paradoxically led to lower vegetative growth. The results are consistent with Went's data and recent studies such as those in Dougher and Bugbee (2001). The study compared the growth of lettuce (*Lactuca sativa*) seedlings under metal halide and high pressure sodium lamps. When conditions were compared, appreciable differences in dry mass, leaf area, and chlorophyll content were noted. However, the light conditions were identical in terms of calculated phytochrome photoequilibrium, blue light, red light, far-red light, and relative ratios between them. The only difference was a significant component between 580–600 nm.

The authors conclude that this band of wavelengths generates negative effects on plant growth, in agreement with the green light data presented in Went (1957) and Klein *et al.* (1965). These reports present a common theme of a negative role for 500–600 nm light in plant growth.

Green light and tropism

Blue light, guides the placement of plant organs, the position of plant organelles and gene expression associated with light capture (Briggs and Christie, 2002; Spalding and Folta, 2005). This suite of responses is mediated by the blue-light-sensing phototropins, as the name implies, the central receptors regulating plant movement. However, green light activation of early phototropic response has also been observed (Steinitz *et al.*, 1985). In *Arabidopsis* and lettuce seedlings, green light-induced phototropic curvature, yet it required substantially more time and a ten times greater dose to generate a response equivalent to a given blue effect.

The authors explicitly state that the green response is due to the blue light receptor mediating phototropism (Steinitz *et al.*, 1985), later designated as phot1. This conclusion was verified genetically when green phototropic effects were absent in *phot1* mutants (Liscum and Briggs, 1996), indicating that phototropism was induced by the extremely minor green absorption sensed through the sensitive phototropin receptors. Also supporting this conclusion was the observation that green light responses were more active as wavelengths approached the blue portion of the spectrum. 510 nm light was more effective than 520 nm and 550 nm light required substantial irradiation to induce the same degree of curvature-induced shorter wavebands of green (Steinitz *et al.*, 1985). These data also argue that the green phototropic response is attributable to phototropin's extreme sensitivity to light, or possibly blue light contamination present in the green light treatments. However, analysis of the photocycle of the oat phot1 LOV2 domain indicated that blue light activation drives formation of a green and red absorbing FMN triplet state that is extremely transient, in the order of nanoseconds (Swartz *et al.*, 2001; Kennis *et al.*, 2003). Due to their short-lived nature, it is unlikely that these species would play any kind of meaningful role in phototropin activity. The work by Steinitz *et al.* (1985) utilized extremely careful illumination conditions, combining narrow-bandpass filters and sharp cutoff filters with little to no transmission below 500 nm. Together, the action spectrum of phot responses and careful experimental conditions suggest that the topic may benefit from contemporary analysis.

Diageotropic growth of roots is mostly controlled through phytochrome activation. Klein (1979) definitively tested light-driven root growth habits in curly cress

(*Lepidium sativum*), a species where positive gravitropic responses are not phytochrome dependent. In this study, green light was shown to slow geotropic root curvature with a discrete maximum at 546 nm. Wavelengths shorter than 520 nm or greater than 580 nm had no measurable effect on slowing normal growth progression. Reversal of green light-induced inhibition was tested using a series of wavelengths between 590 nm and 670 nm. The data show that light at 620 nm was most effective at reversing the green light response, and that irradiation near the phytochrome peak absorption (660 nm) had almost no effect. Blue light did not influence these responses; therefore these data are consistent with a cryptochrome-independent mechanism in contemporary interpretations.

Recently, a study of leaf inclination by Mullen *et al.* (2006) analysed the genetic basis of leaf inclination associated with shading, sensed as a decrease in the red to far-red ratio (Fig. 1). The study showed that changes in leaf inclination were directed by phyA, phyB, and phyE. However, multiple-order phytochrome mutants still maintained the response in white light. Surprisingly, the green light component of white light was shown to induce the response in the *hyl* and *phyAphyBphyE* mutants, indicating a separate mechanism for green guiding of leaf inclination. The authors tested the *npq1* mutant due to its effects on green interruption of stomatal opening (Frechilla *et al.*, 2000; detailed below) and it responded as the wild type. However, the *npq2* mutant, a mutant required for ABA synthesis, was unable to induce this response. Application of ABA restored normal leaf angles, indicating that a change in ABA levels may be the terminal effect of this green light response.

Enter heliochrome

USDA scientist Takuma Tanada studied photobiological responses in plants. Data from many of his studies pointed to the existence of a far-red/green reversible receptor acting complementary to phytochrome termed 'heliochrome' (Tanada, 1997). The evidence for heliochrome arose from studies where responses induced by far-red light (≥ 710 nm) were completely negated by the application of comparatively small amounts of green light (550 nm). Red light (660 nm) was completely ineffective in negating the far-red responses, demonstrating that the observed effects were not due to Borthwickian red/far-red reversible phytochrome activity.

In one study red, far-red, and green relationships were tested by monitoring the closing of *Albizia julibrissin* pinnules (Tanada, 1982). *Albizia* pinnules exhibit nyctinastic closure, and closing can be delayed by far-red light of 710–730 nm. The delay induced by far-red light at substantial fluence rates ($18\text{--}43 \mu\text{mol m}^{-2} \text{s}^{-1}$) could be completely negated by co-illumination with dim green

light ($0.01\text{--}5 \mu\text{mol m}^{-2} \text{s}^{-1}$). Green treatment alone had no effect, yet alternating different combinations of red, far-red, and green pulses showed that green and far-red could toggle the response. Most importantly, red light at 660 nm had no effect, indicating that the observed responses were not classical phytochrome effects (Tanada, 1982). Later, the phenomenon was tested using bright flashes of light. Here, blue light (450 nm) could delay closing, similar to far-red light (Tanada, 1984b) and green light could completely negate the blue light effect. Tanada concluded that the far-red absorption state of heliochrome must also be blue sensitive.

Similar results were reported in studies in *Brassica campestris*, where a far-red day extension induced prolific flowering (Tanada, 1984a). Co-irradiations with increasing fluence rates of red light could not reverse the far-red induction. Rather, minor illumination with green light caused a fluence-rate-dependent decrease in the number of plants committing to floral habits. The author also shows that inductive day extensions from a 710 nm pulse could be completely negated with a 550 nm pulse, and that the 550 nm inhibition could be reversed with a 710 nm flash. As in previous studies (Tanada, 1982), 710 nm light was more effective than 730 nm (the peak of Pfr absorbance) and 750 nm had no effect.

Tanada is probably best recognized for the 'Tanada effect', a discovery made while rinsing experimental glassware. Some excised oat and mung bean root tips used in experiments would adhere to beakers, others would not. Binding was shown to be rapid (occurring within 30 s), and red and far-red reversible, indicating phytochrome response (Tanada, 1968). These observations were consistent with the hypothesis that phytochrome induced ionic changes as primary steps in the signalling process. This effect was later revisited in soybean experiments by measuring light-induced changes in potential using an electrometer (Tanada, 1983). As seen in earlier experiments red/far-red (660/760 nm) reversibility of potential changes indicated a clear role of phytochrome. However, irradiation with 710 nm light led to changes similar to those induced by 660 nm. This induction could be completely negated with a subsequent pulse of green light, but could not be reversed by red light. Interpretation of these data is problematic because blue light was not tested, nor were green effects tested extensively. However, this study presented evidence that green light could negate an effect of a far-red treatment that was uncoupled from phytochrome activity. Its far-red nature argues against a cryptochrome component, as the redox states of cry chromophores have negligible absorption in the far-red.

The data supporting the heliochrome hypothesis could be explained with today's understanding of plant photosensing if two assumptions are made. First, the effects are cryptochrome driven and reversed by green light acting

via the neutral semiquinone chromophore. Also, far-red effects would have to be imparted through phyA and parallel the blue effects, much like phyA and cry2 together promote CONSTANS stability leading to flowering (Valverde *et al.*, 2004). However, far-red/green effects are difficult to reconcile unless the green form of cryptochrome overrides or causes degradation of phyA. These are cumbersome models to test, but remain the only way to resolve these data, as the original work was always careful yet unknowingly incomplete.

When considered together, the far-red/green reversibility of pinnule closing, flowering, and ionic results are perplexing. The experiments were performed in sensitive systems, measuring the effects of finite treatments over short time-courses. Dose–response relationships were often demonstrated and the physiological responses observed were not ambiguous. In 1997 Tanada proposed that heliochrome was a haem-based receptor, and today this possibility could be tested using haem oxygenase mutants. Even with the benefit of hindsight, Tanada's work presents another interesting case where green light exerts specific effects on plant responses that are not easily explainable without invoking complex interactions of dichromatic states within known sensors or some effect of a novel receptor.

Green light opposes stomatal opening

Zeiger and colleagues (Frechilla *et al.*, 2000) demonstrated that a brief pulse of green light could preclude blue-light-mediated stomatal opening in *Vicia faba* epidermal peels. Closer evaluation of this phenomenon revealed the blue-green reversible dichromaticity, as the quality of the last pulse of light delivered dictated the physiological response observed. If green was given followed by blue, then the stomates opened, whereas if the pulsed sequence was green, blue, green the stomates remained closed. This group went on to show that this unusual stomatal response was dose-dependent with the most significant effect at 2:1 green:blue (Talbot *et al.*, 2002). An action spectrum revealed 540 nm light to be the most effective wavelength for reversal (Frechilla *et al.*, 2000). In addition, this response persisted in a background of red light which indicated the blue-green pathway was separate from the previously described phytochrome or photosynthesis-driven pathways that influence stomatal aperture. Later, Talbot *et al.* (2002) observed this blue-green effect in a diverse suite of plant species suggesting that the effect is present throughout the plant kingdom.

The authors suggested that the entity that absorbs and responds to blue and green light may toggle between active and inactive states similar to the phytochrome Pfr and Pr states (Frechilla *et al.*, 2000). One candidate put forth was a carotenoid, zeaxanthin, which was previously

described as a candidate for blue-induced stomatal opening (Frechilla *et al.*, 1999). The action spectrum for the response matched the absorption spectrum for a carotenoid, only red shifted 50 nm. Similar results were observed by Eisinger *et al.* (2003) who showed that a green light pulse could negate the effect of ultraviolet light on stomatal opening.

Later the phototropins were clearly implicated in the control of stomatal aperture (Kinoshita *et al.*, 2001). In combination with the studies by Zeiger's group, this finding suggested that the phototropins may be blue-green reversible, yet analyses of phototropin LOV domain absorbance did not support this hypothesis, as absorbance in the green was found to be extremely transient, on the order of a few nanoseconds (Kennis *et al.*, 2003; Swartz *et al.*, 2001).

This discrepancy was addressed by Talbot *et al.* (2003) with a genetic study in *Arabidopsis* mutants. Analysis of *phot1phot2* double mutants and *npq1*, a mutant with a lesion in violaxanthin de-epoxidase which does not accumulate appreciable amounts of zeaxanthin (Niyogi *et al.*, 1998), revealed that NPQ1 but not phot1 or phot2 was required for the blue-green stomatal response pathway. In this study, the authors examined various mutants and red and blue induction of stomatal opening and associations with far-red and green reversal. The *phot1phot2* and *npq1* mutants behaved as wild type in terms of the red/far-red stomatal response. Further investigation revealed that *phot1phot2* double mutant stomata demonstrated viable red-far-red and blue-green reversibility, however *phot1phot2* mutants required more blue light to accomplish blue-induction of stomatal opening. These data confirm that phot1 and/or phot2 regulate the blue stomatal response; however, they are not the photoreceptors mediating the blue-green reversible facet of stomatal opening. On the other hand, *npq1* mutants lacked the ability to respond to blue light, but maintained red-induction and far-red reversal. These tests demonstrated the existence of independent stomatal regulatory pathways, defined the discrete roles of NPQ1, phot1, and phot2 in the response, and further delineated the role of NPQ1 in the blue-green reversible guard cell action (Talbot *et al.*, 2003).

Later work by this group showed that green light influences in this pathway involved a circadian component, as it was most prevalent in the morning (Talbot *et al.*, 2006). In this study the authors add more evidence to previous work demonstrating NPQ1's involvement in the blue-green stomatal pathway, in that *npq1* does not respond to blue or green light, while *phot1phot2* double mutants respond as wild type. This work, in conjunction with other analyses, indicates that the effects of green light are conditional and that the use of mutants and specific light treatments is required to delineate specific pathways. An additional report has demonstrated a role

for cryptochromes in guard cells that is distinct from phot effects (Mao *et al.*, 2005). In consideration with the green-blue toggling of cry2 accumulation and response (Bouly *et al.*, 2007) it will be important to test if cryptochromes are relevant to the blue-green reversibility of stomatal opening.

Green light effects on leaf growth and stomatal conductance

The effects of green light on stomatal opening noted by Zeiger's group were extended to whole plants by NASA scientists. Plant growth in artificial environments remains a key provision to long-term space colonization. Therefore NASA scientists have explored the effects of combinatorial light conditions on plants. Many of these studies simply focused on the effects of narrow-bandwidth red and blue sources compared to conventional sources (Brown *et al.*, 1995; Goins *et al.*, 1997; Yorio *et al.*, 2001). One central concern emerged when plants were grown under some light conditions. Plants grown under red and blue LEDs appeared black or purple rendering it difficult to monitor plant growth and health in the artificial state. Also, miscoloured plants are not as visually appealing to a potential crew (Kim *et al.*, 2004a).

With the goal of making plants appear green NASA scientists assessed the effects of green light supplementation to a red and blue background, and discovered that addition of this allegedly benign light quality generated conspicuous effects. These experiments differed from those performed by Went and Klein in that these kept PPF constant and varied the proportion of green light added. This approach has the advantage of keeping metabolism static, yet the disadvantage of skewing activation of photosensory networks that contribute to developmental responses. These studies also use different species and developmental states relative to earlier studies. For this reason the results need to be considered independently of the previously described work.

In these reports the effects of combinatorial red, blue, and green (RB+G) light treatments on leaf growth and stomatal conductance in lettuce were compared to red and blue (RB) alone (Kim *et al.*, 2004a, b). Green light supplied by green fluorescent lamps was added to a background of red and blue LED light. There was very little (if any) far-red light which is important for discounting potential phytochrome interpretations. The authors discovered that lettuce plants grown in RB+G treatments displayed leaves with larger specific leaf area and less thickness compared with RB alone (Kim *et al.*, 2004a). Also, plants grown under RB treatments demonstrated higher stomatal conductance when compared with those under RB+G, with the lowest stomatal conductance reported in plants grown under green fluorescent lamps

alone (Kim *et al.*, 2004b). In addition, while stomatal conductance was greater in cool white fluorescent treatments than in RB+G, the dry mass of the plants was greater in RB+G implying the weaker stomatal conductance did not negatively affect carbon assimilation (Kim *et al.*, 2004b). Plant dry mass was greatest under RB+G treatments (where 24% of the spectrum was broadband green light) when compared with RB, the opposite of the effects noted by Went (1957; Fig. 2). However, these results do agree with previous findings that plants grown in RB+G treatments displayed larger specific leaf areas than those grown under RB treatments (Kim *et al.*, 2004a). These experiments demonstrate that supplemental green affects plant physiology in conditions where red and blue systems are saturated. It remains to be seen if these effects are cry-dependent or cry-independent, as they were performed in species where photoreceptor mutants are not yet available.

Early stem elongation

The identification of green-blue reversibility in early plant responses led to the assessment of green effects, if any, on early stem growth rates. The elongating hypocotyl is a dynamic organ that adjusts its growth rate to match prevailing conditions (Parks *et al.*, 2001) so it is an ideal system to identify subtle contributions of light-sensing systems. High-resolution image capture techniques were employed with *Arabidopsis* mutants to identify discrete roles of phytochromes (Parks and Spalding, 1999), cryptochromes (Folta and Spalding, 2001), and phototropins (Folta *et al.*, 2003a) in acclimation to the early light environment.

Dark-grown seedlings were given a pulse of blue light followed by a pulse of green. A characteristic phot response was observed, as seedlings exhibited a normal first-phase of growth inhibition as described (Folta and Spalding, 2001; Folta *et al.*, 2003a). However, within minutes, and only after receiving a green light pulse, seedling growth would accelerate to 150% of the dark rate. The effect of green was unlike any previously described, as plants would elongate at a rate that exceeded their dark (and presumably most rapid) rate. This unusual green-induced increase in stem elongation rate was later examined in great detail. Single, etiolated seedlings were tested for the elongation response to a brief green light pulse. Within minutes of a dim-green-safelight-quality light pulse the dark-grown seedling would elongate faster than it would elongate in complete darkness (Folta, 2004). The response was dose-dependent, obeyed the Bunsen-Roscoe Law of Reciprocity, and was observed in response to a pulse barely detectable by eye. Green-pulse-induced growth acceleration was transient, with growth rates eventually braking back to those exhibited by dark-grown

seedlings within 1 h. Most importantly, the green light-mediated growth induction persisted in *cry*, *phy*, *phot*, and *npq1* mutants, indicating that the response was mediated by redundant function between known receptor classes or that it was initiated by a novel light sensor. Additional experiments indicated that the green response was maintained in a background of dim red light, suggesting that phytochrome was not the receptor because increasing phytochrome activation with green light would engage growth restriction, not elongation. The effect of green persisted in a dim red and blue background (Folta, 2004; SA Maruhnich and KM Folta, unpublished observation). These findings indicated that green light was acting antagonistically to red and blue light, as 'safelight' doses of light could induce responses contrary to normal photomorphogenic development. Similar studies later attributed the long-term blue-green reversibility to cry receptors (Bouly *et al.*, 2007) and will be discussed further below. Together, these studies delineate cry-independent and cry-dependent mechanisms associated with stem elongation and acclimation to the light environment.

Green light down-regulates plastid transcript accumulation

The characterization of green-induced stem growth suggested the possibility that a novel green-light-sensing system was initiating a response to a subtle environmental cue. The study also provided, with great resolution, a time point to perform transcriptome analysis in an attempt to understand the molecular basis of green light response and possibly green light signalling. An identical approach was fruitful in identifying genes associated with cryptochrome-mediated growth inhibition (Folta *et al.*, 2003b). The previous stem growth rate study demonstrated that green light caused an increase in stem elongation that was induced by a short, single pulse of dim green light and was well established, if not complete, by 60 min (Folta, 2004). Whole *Arabidopsis* genome microarrays were used to compare the transcriptomes of dark-grown seedlings to seedlings treated with a short, single pulse of green light in a quantity sufficient to simulate optimal stem elongation.

The microarray data presented some anticipated results (Dhingra *et al.*, 2006). For instance, a suite of genes known to be controlled by phyA (including *Hy5*, *Pks1*, and *ELIP*) was induced, as though the plants were illuminated with red or far-red light (as in Tepperman *et al.*, 2001). This result was expected and validated that the phytochrome system was responding correctly. These data implied that changes not attributable to phytochrome would have a high likelihood of being relevant to green light sensing or response. While phytochrome transcripts increased, a number of chloroplast transcripts (including

several previously shown as light inducible) sharply decreased after a pulse of green light. An examination of candidate transcripts showed that the response was rapid (occurring within 15–30 min), sensitive (with a threshold $<10^1 \mu\text{mol m}^{-2}$) and obeyed reciprocity. Parallel effects of green light were observed in tobacco (*Nicotiana tabacum*), indicating that this response was not confined to *Arabidopsis*. Moreover, the down-regulation of plastid transcripts persisted in all photomorphogenic mutant backgrounds tested, suggesting that the green signal is possibly dependent upon a novel sensory scheme. Taken together, an increase in stem elongation and coincident down-regulation of plastid transcripts may represent the plant taking on characters associated with a semi-skotomorphogenic strategy. This state would allow conservation of resources while simultaneously producing changes in growth, facilitating capture of favourable illumination.

A connection to plant biomass

Sommer and Franke (2006) presented evidence that treatment of seeds with green lasers led to enhanced fresh weight of plants at the time of harvest. The authors are not plant biologists. Instead their previous publication stream addresses laser light effects in speeding wound healing (Sommer *et al.*, 2001) and increasing cell vitality in animals (Sommer *et al.*, 2002). Here they expand their examination of high-fluence rate light effects on biological systems to plants and identified that dry carrot, radish, and cress seeds treated with a green laser or intense green LEDs produced plants with significantly greater biomass than control plants (no light pretreatment). All plants shared equal conditions after the seeds were irradiated. All seedlings emerged at the same time, so the differences observed later could not be simply attributed to phytochrome-induced enhancement of germination in laser-treated seeds. Mature radishes and carrots from laser-treated seeds were twice as large as controls (Sommer and Franke, 2006). The authors were more intrigued with the outcomes than the causes and note that this may be a way to hasten the growing season.

While interesting, statistical rigour was thin and the authors also did not account for the possible explanation that phytochrome was activated and may have led to the advanced developmental state of the irradiated seedlings. With the head start, seedlings might establish faster and more completely than their non-irradiated siblings. However, follow-up experiments compared red to green laser treatment of *Arabidopsis* seeds. Green-treated seeds germinated and emerged later than red-treated seedlings, yet still exhibited a larger end-point root phenotype, suggesting that the pre-illumination did not drive a phy-induced enhancement of early development (A Sommer, personal communication).

These curious conclusions constitute a cause to question the potential link between early illumination and long-term phenotypes. These findings are completely contrary to the very nature of plant development, where ambient environmental cues generally supercede innate signals. However, it remains conceivable that some sensory input may hard-wire the young seedling to anticipate and prepare to exploit an ample light environment before emergence. Additional study of this phenomenon should be conducted, as the substantial changes in biomass may be of great benefit in increasing crop production, the generation of plant mass for biofuels, and/or the introduction of additional plant mass into planting fields as green fertilizer.

Cryptochrome-dependent green light effects

Shortly after characterization of *Cry1* as the gene encoding the protein with potent roles in blue light signalling, attention turned to its further description as a photosensor. In 1995, two reports described association between CRY1 and two potential chromophores, FAD, and a second chromophore, 5,10-methylenyl tetrahydrofolate (Lin *et al.*, 1995b; Malhotra *et al.*, 1995). Studies in plants and fungi since the late 1960s suggested that flavoreceptor signalling would involve changes in the redox state of the chromophore. These suspicions have since been confirmed, as a significant amount of cryptochrome produced in insect cells exists in the semi-reduced form (Lin *et al.*, 1995b). Biological consequences of the flavosemiquinone have been identified in *Phycomyces* (Galland and Tolle, 2003) and *Arabidopsis* (Bouly *et al.*, 2007). Figure 3 depicts a model of the redox states of the flavin chromophore and how they relate to photosensor activity. Dark-grown plants would contain the fully oxidized chromophore (FAD) that would absorb blue light, converting to a semi-reduced (FADH•) or fully reduced (FADH⁻) chromophore. The semi-reduced form is the biologically active, yet green-absorbing form. Addition of green light drives full reduction and inactivation (Bouly *et al.*, 2007). The degree of biological activity is

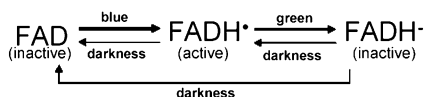


Fig. 3. The proposed photocycle for plant cryptochromes. In darkness the cryptochrome flavin chromophore exists in the oxidized form (FAD), rendering the photoreceptor inactive and stable, accumulating to high levels. Stimulation by blue light drives reduction of FAD to FADH•, the active signalling state (and green absorbing state) of the receptor. Illumination with green light generates the fully reduced form of the chromophore (FADH⁻) that inactivates the receptor. Dark reversion returns the flavin from all reduced forms to the oxidized, blue absorbing state. (Figure adapted from Bouly *et al.*, 2007 and is reproduced by kind permission of the American Society for Biochemistry and Molecular Biology.)

determined by the relative quanta of blue and green light, resulting in a pool of semi-reduced, fully-reduced, and fully-oxidized chromoproteins.

This theoretical model is supported by new biological evidence. Studies of cryptochromes in insect cells demonstrated that the initial light reactions in cryptochrome signalling depend on electron transfer from conserved tryptophan or tyrosine residues to reduce a flavin chromophore (Giovani *et al.*, 2003), in accordance with models derived from photolyases (except that the latter possess the reduced flavin as a chromophore). The action spectrum for cry1-mediated inhibition of hypocotyl elongation shows a peak at 450 nm (Ahmad *et al.*, 2002), consistent with the oxidized flavin chromophore when catalytically active. Bouly *et al.* (2007) show that green light (563 nm) can reverse the effect of blue light on hypocotyl elongation in developing seedlings, consistent with previous reports of green reversal of blue and red irradiation (Fig. 8 in Folta, 2004). Bouly *et al.* (2007) then show that this effect is cry-dependent, supporting the green-blue reversibility model. The cry2 receptor has been shown to be light-labile (Ahmad *et al.*, 1998). The Bouly *et al.* (2007) report goes on to test CRY2 accumulation in response to pulses of blue light, and blue light followed by finite pulses of green light. The results are most convincing, as they show that blue light degradation of cry2 can be reversed by a short, single pulse of green light, suggesting that the semi-reduced, active state is transient and subject to adjustment by neighbouring wavelengths. These data represent excellent evidence of a dichromatic modulation of cryptochrome activity.

Since cry2 has a profound effect on controlling flowering time (Guo *et al.*, 1998; Valverde *et al.*, 2004), the green stabilization of CRY2 could have potential ramifications in controlling the transition to flowering. This was tested in *Arabidopsis* plants grown on short day (non-inductive) conditions followed by transfer to blue, green, or simultaneous green and blue light conditions (Banerjee *et al.*, 2007). Plants transferred to blue light flowered earlier than those remaining in white light cycles. However, when plants were moved to blue and green co-irradiation, the addition of green light negated the effect. The green light inhibition of flowering agrees well with earlier studies where removal of green light enhanced flowering in marigold (Klein *et al.*, 1965) and addition of green light suppressed flowering in carnation and lettuce (Vince *et al.*, 1964). In a mechanistic context, the levels of FT transcript, an inductive output of the photoperiod pathway were assessed, and were shown only to accumulate in blue light conditions, again illustrating the antagonistic effects of green light (Banerjee *et al.*, 2007). It will be interesting to see how CONSTANS localization and stability are affected by green wavebands, as this central regulator is strongly dependent upon stabilization imparted through cry2 (Mockler *et al.*, 1999; Valverde *et al.*, 2004).

The recent characterization of a green-dependent cry sensing state is an exciting finding and leads to the following questions: Is there a specific effect of the fully reduced (green induced) flavin in cry interactions with other signalling components such as phys or COP1? What are the effects of green light on the many cry-regulated plant responses? How do green and blue differ in terms of inducing differential presentation of active domains of the cry receptor? These questions will probably be addressed in ongoing research.

Is the photosensor class complete?

Clearly, green wavebands play a potent role in regulating plant growth and development, and at least a portion of the responses are dependent on cryptochromes. The extent that blue-green cryptochrome relations affect plant responses promises to be an attractive area of further research going forward. However, the sensor(s) affiliated with cryptochrome-independent responses remains to be identified. Several candidates have been suggested by previous work. Zeiger and colleagues have proposed zeaxanthin, a carotenoid which is required for the blue-green reversible stomatal regulating pathway is a green receptor which absorbs both blue and green wavebands and toggles between an 'active' and 'inactive' state (Frechilla *et al.*, 2000).

Other intuitive candidates provide a basis for further investigation. An abundant flavoprotein was isolated from the membranes of *Cucurbita pepo* and *Phycomyces* (Hertel, 2005). The protein was later shown to have homology with type-1 aquaporins (Lorenz *et al.*, 2003). *In vitro* analyses indicated that the protein binds flavin and that the binding can be reversed with chemical or blue light treatment. Although the *in vitro* work by Lorenz *et al.* (2003) presents no evidence of light effects above 500 nm, the protein remains an interesting candidate as a green light receptor *in vivo*. Such a protein would be a logical candidate for green-induced stem elongation (Folta, 2004) as such robust short-term growth accelerations would likely require a rapid change in turgor.

CRY3 (or CRY-DASH) is another flavoprotein with high local sequence similarity to cryptochrome photoreceptors and photolyases. Recently, this protein has convincingly been shown to act as a photolyase specific for single-stranded DNA (Selby and Sancar, 2006), suggesting that it would probably not be functioning as the green photosensor for orphan responses. However, since CRY-DASH localizes to the chloroplast (Kleine *et al.*, 2003), the CRY1 protein from *Vibrio cholerae* binds RNA, and one of the green light responses probably requires RNA metabolism (Dhingra *et al.*, 2006) *cry3* mutants should be tested for roles in the plastid transcript response. In addition, CRY3 is the only photoreceptor

up-regulated in green light (Dhingra *et al.*, 2006; and their supplementary data).

The existence of other sensors is implied by the literature. GCR1 (G-protein coupled receptor 1) in *Arabidopsis thaliana* has the strongest homology to the major photoreceptor of animal vision in terms of structural topology (Colucci *et al.*, 2002). GCR1 has been found to interact with the alpha subunit of the heterotrimeric G-protein in plants (Pandey and Assmann, 2004), and it has recently been shown to be required for blue-low-fluence-mediated *Lhcb* induction in etiolated seedlings (Warpeha *et al.*, 2007). G-proteins have been pharmacologically (Neuhaus *et al.*, 1993) and functionally (Okamoto *et al.*, 2001) linked to light responses. Since mutants in these components are readily available they could be tested in the suite of green light assays. Studies of potential chromophores for plant light receptors identified *all-trans* retinal in tomato extracts (Lorenzi *et al.*, 1994), the same isomer later shown to be most active in fungal and microbial opsins. Other evidence identifies retinal-binding proteins in plants and implicates retinoids in blue-light regulation of stomatal aperture (Paolicchi *et al.*, 2005). Could there be a link between these findings and the carotenoid-dependent blue-green reversibility observed in guard cells? Could heliochrome, a hypothetical far-red/green reversible haem-based receptor (Tanada, 1997) still be identified?

Since green light-associated phenotypes are subtle and often require specialized equipment to visualize them, large genetic screens are not practical in identifying potential mutants. Yet, based on the literature, the loss of a non-cryptochrome green light sensor would be predicted to exhibit a light-dependent, hyper-photomorphogenic phenotype. Such mutants were isolated by Pepper *et al.* (2001) in mutant screens performed under low fluence rate white light and green light. Complete characterization has not been reported. It is likely that components of the green sensing system have been isolated from the many screens performed under red, blue, or white light. In support of this notion, many photomorphogenic signalling mutants have been well studied yet do not fit conveniently into cry and phy pathways. These represent an excellent starting point for further tests, now that several cry-independent green light assays have been defined.

Conclusions

Recent findings of cry-dependent and cry-independent green photoresponses suggest that green, in addition to red, far-red, blue, and UV sensory mechanisms, monitor and adjust plant growth and development. For the most part, the recent findings mesh well with central themes from older studies performed before the advent of molecular-genetic tools and modern techniques. One theme presented throughout this review is that the effects of green light tend to reverse the processes established by red and/or blue

light. In this way, green light may be functioning in a manner similar to far-red light, informing the plant of photosynthetically unfavourable conditions. Although seemingly counterintuitive at first, these conclusions make sense in the context of normal plant growth in natural settings. In terms of basic science, together these findings remind us that nature tends not to ignore a conditional environmental input and that inductive biological systems often have antagonistic systems that counter their progression. In this way plants use the full spectrum and the relative ratios of energies within to adjust their form, composition, and physiology to best exploit prevailing conditions.

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