16 • Photosynthetic responses to radiation

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16.1. RADIATION AND PLANTS: AN INTRODUCTION TO PHOTOBIOLOGY

16.1.1. Basic characteristics of solar radiation

Among the factors affecting plants, solar radiation is perhaps the most heterogeneous in space and time. Important parts of solar radiation provide energy for photosynthesis and serve as signals in photoregulation of plant growth and development. The sun radiates energy in the spectral range from 280 to 4000 nm, with a maximum in the blue-green (480 nm; Fig. 16.1). Within the PAR, solar radiation peaks at ca. 590 nm (Fig. 16.1). Solar radiation can be segregated into direct solar radiation and diffuse sky radiation, which reaches the ground after multiple scattering on atmospheric particles and clouds, reflection from the ground surface and additional scattering in the atmosphere (Ross, 1981).

The widespread, albeit vague, term light is used for the portion of the electromagnetic spectrum in the vicinity of visible light (Kohen *et al*., 1995). Many past ecological and physiological studies were based on measurements that represent the stimulation of the human eye by radiant energy, a measure called illuminance and expressed in foot-candles (English system) or luxes (metric system). The human eye is most sensitive in the green spectral region, centered around 550 nm, whereas any quanta in the spectral region of 400–700 nm have enough energy to drive photosynthesis, so illuminance is obviously not well suited for plant science.

With further developments in understanding the way solar radiation drives photosynthesis, PAR has been used extensively in plant biology and ecology. Roughly half of the solar radiation is in the region of PAR and the rest in the NIR radiation (Fig. 16.1). PAR in W m^{-2} is measured by radiation sensors (pyranometers) equipped with specific filters to remove ultraviolet (UV) and IR spectral parts (Pearcy, 1989). In most countries, PAR is defined as radiation in the spectral interval 400–700 nm, whereas in the former Soviet Union and socialist countries, PAR was defined as radiation between 380–710 nm (Ross and Sulev, 2000). This discrepancy leads to measurements 5–7% higher when the extended interval is considered instead of the most common one.

However, the amount of quanta that ultimately drives photosynthesis can be different for the same PAR values depending on the spectrum of light, e.g., diffuse solar irradiance enriched by blue quanta with higher energy contains fewer quanta at a given PAR than direct solar irradiance, with more-uniform spectral distribution. At low sun elevations, light is enriched by orange, red and FR photons (end-of-day effect) with lower energy, and accordingly there are more quanta at a given PAR. Different light sources used in plant science also have different quantum/energy conversions. Therefore, photosynthetically active quantum-flux density (PPFD, μmol m^{-2} s^{-1}) is currently the most widely used quantity to characterise plant-light availability. The most reliable but at the same time costly way of estimating PPFD is by measuring the radiation with a spectroradiometer and integrating spectral irradiance over the region 400–700 nm. The most common estimations of PPFD are conducted by quantum sensors based on silicon photodiodes, equipped with specific filters to remove IR- and UV-spectrum parts (Pontailler, 1990).

Solar radiation reaches the top of the Earth atmosphere at a rate of 1396 W m^{-2}, the so-called solar constant. The solar 'constant'varies about 5% depending on the distance between the sun and the Earth, and on the activity of the sun. On cloudless days, only 800 to 1200 W m^{-2} of total radiation reach the Earth's surface owing to radiation absorption by the atmosphere. The light environment in a given site is then a consequence of the latitude of the site, time of the day, neighbouring plants and surrounding objects casting shade, and of a number of properties of the low and variable

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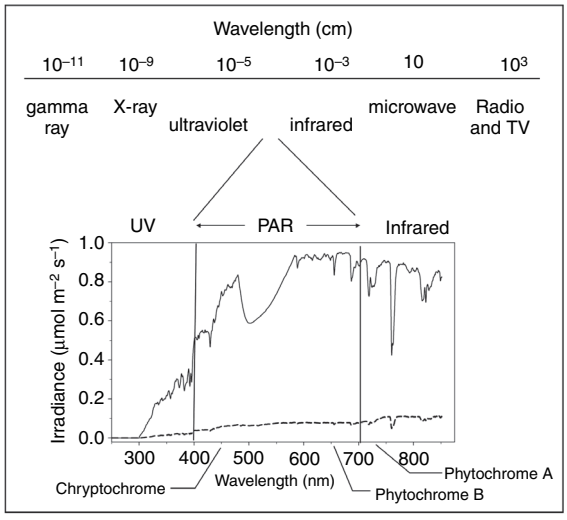

Fig. 16.1. Electromagnetic spectrum of solar irradiance with detailed view of the region relevant for photosynthesis and plant performance (UV, ultraviolet; PAR, photosynthetically active radiation; and infrared). The detailed sunlight spectrum for the 300–800 nm region corresponds to a typical clear summer day at 40 degrees latitude both in the open (continuous line) and in the understory of an evergreen rainforest in Chile (Puyehue, 40° 39'S, 72° 11'W; dashed line). Plants are particularly sensitive to irradiance of certain wavelengths owing to specific phytochromes and cryptochromes (main photoreceptors indicated in the graph, see text for further details).

atmosphere, mainly cloudiness and transparency. All these factors affect the radiation spectra, the intensity and direction of beam radiation, the distribution of the radiation between beam and diffuse components and the duration of the photoperiod. The amount of PPFD in total solar radiation is a highly variable quantity. For instance, idealised conversion coefficients (PPFD/Total solar radiation, µmol/J) of 1.814 for global, 1.758 for direct, 2.127 for diffuse and 0.462 for reflected solar radiation have been proposed (Ross and Sulev, 2000), but these coefficients differ under different environmental conditions.

Many of the factors altering the amount of total solar radiation that drives leaf energy balance and evaporation, and PPFD that drives photosynthesis have been considered in a number of models to estimate the radiation below and/or reflected by a canopy, but theoretical estimations and actual measurements often do not coincide well (see references below). For instance, the sun disc is completely blocked by an object at a theoretical distance of 108 times the object diameter, but empirical data show that this distance is reduced to 50–70 times the diameter of the object. When direct sunlight is blocked by clouds or by a canopy, light reaching a plant comes from the entire sky hemisphere (diffuse light); in such conditions, the shadow of an object vanishes at a distance equal to its diameter (Horn, 1971; Valladares and Niinemets, 2007). Directionality or the lack of directionality and the so-called penumbral effects are important sources for discrepancies between the theory and the reality of a particular light environment (Oker-Blom, 1984; Smith *et al.*, 1989). In particular, it is very hard to model stochastic events, such as changes in beam irradiance, owing to movement of clouds and movement of canopy elements, e.g., as a result of wind. Several simplifications used in modelling diffuse irradiance, such as uniformly overcast sky conditions or standard overcast sky conditions (the latter accounting for brighter sky towards the sun) are very crude simplifications (Hutchison *et al.*, 1980; Spitters *et al.*, 1986). Nevertheless, long-term light estimates, for instance for the entire growing season, can generally be estimated rather well even with crude assumptions (e.g. Roderick, 1999).

16.1.2. Solar radiation within vegetation

16.1.2.1. RADIATION QUANTITY

One of the most important factors affecting the light environment is the presence of plant canopy that harvests the light, but also modifies the intensity and spectral quality of the penetrating radiation. Accurate description of the light environment in a forest understory is more complex than in the open, not only because of its intrinsic spatial and temporal heterogeneity, but also because of forest-type specific structural features that alter the correlations between light environment and aggregated-canopy characteristics, such as canopy height, density and LAI (the amount of leaf area per unit ground area). PAR in the understory ranges from 50–80% of full sunlight under leafless deciduous trees to 10–15% in even-aged pine stands, 2.5% in closed spruce canopies, 0.2–0.4% in dense beech forests and even less than 0.1% in certain tropical rainforests (Barnes *et al.*, 1998). These values vary in dependence on factors such as wind speed and cloudiness. On windy days, more beam irradiance can penetrate into deeper canopy horizons (Tong and Hipps, 1996), whereas on cloudy or overcast days, the relative percent of radiation transmitted by forest canopies is higher than on clear days (Endler, 1993; Federer and Tanner, 1966). It is well known that the percentage of sunlight available in a forest canopy decreases exponentially as the cumulative LAI (LAI_{cum}) increases (e.g., Baldocchi and Collineau, 1994; Cescatti and Niinemets, 2004). Initially, simple exponential relationships following the Lambert-Beer law were proposed

(Monsi and Saeki, 1953; now an English translation of this classical paper is available Monsi and Saeki, 2005) describing the relationship between canopy to-incident radiation (Q_0) and any level inside the canopy, Q, as $Q=Q_0\exp(-k\mathrm{LAI}_{\mathrm{cum}})$, where k is the extinction coefficient depending on solar angle and leaf angular distribution. This equation assumes that foliage is randomly dispersed. However, foliage in natural communities is often clumped, intercepting less light for any given $\mathrm{LAI}_{\mathrm{cum}}$ than the foliage with random dispersion; the foliage may also be regularly dispersed, intercepting more light than the foliage with random dispersion (Cescatti and Niinemets, 2004). Parameterisation of such differences requires at least one more parameter. In addition, such one-dimensional models often fail in more complex heterogeneous canopies, where three-dimensional models provide a much better but still stochastic description of canopy light environment (Cescatti and Niinemets, 2004). Ray tracing models, describing the location of every foliage element in space and providing the best correspondence between actual and simulated light environment (North, 1996; Pearcy and Yang, 1996) have won popularity, especially for studies of functional significance of plant form (Falster and Westoby, 2003; Valladares and Pearcy, 2000). However, such models require huge (and often impractical) effort to parameterise at stand scale (Niinemets and Anten, 2009), although more-efficient parameterisation routines are currently under development (Casella and Sinoquet, 2003; Cescatti and Niinemets, 2004).

16.1.2.2. RADIATION QUALITY

Light transmitted through a canopy experiences remarkable spectral alterations owing to enhanced capture of photons within the PAR region, and becomes enriched by radiation in green wavelengths and NIR, where leaves absorb radiation inefficiently. Although overall PAR transmittance in a Costa Rican rainforest at solar elevation near the zenith was as low as 0.5%, transmittance in the FR or NIR (ca. 730 nm) was about 4% (Lee, 1987). Spectral changes in transmitted light depend not only on canopy LAI, but also on spectral quality of incident radiation, for instance on cloudiness. On cloudy days, spectral changes in light transmitted through the canopy are minimised (Federer and Tanner, 1966; Barnes *et al*., 1998). In addition, various species alter the light gradient to a different degree. For instance, conifers with thick foliage elements that are essentially optically black in PAR and NIR, and that are also strongly aggregated into shoots with a relatively large gap fraction inside the canopy, do not modify the spectrum to the same extent as deciduous species (Federer and Tanner, 1966; Barnes *et al*., 1998).

According to the colour of light, a total of five basic light environments have been described in terrestrial ecosystems (Endler, 1993; Kiltie, 1993): (1) forest shade characterised by greenish or yellow-greenish light owing to selective absorption of red and blue by vegetation; (2) woodland shade with bluish or bluish-grey light owing to the dominance of the radiation from the sky; (3) small gaps characterised by yellowish-reddish light owing to direct sunlight; (4) large gaps, open or any habitat under cloudy conditions where the light is whitish owing to the combination of sun and sky light, or because of the dominance of the white light radiating from clouds; and (5) any habitat early and late in the day, when the sun is below 10° from the horizon and light is mainly orange, red, FR and, under certain atmospheric conditions, purple.

Although visible light, more specifically PAR, is the most relevant portion of the electromagnetic spectrum for plants, radiation of shorter UV or longer FR wavelengths is important in a variety of signalling responses. Plants have three main photoreceptors: cryptochromes sensitive to blue-light and UV-A (450 nm and <400 nm), phytochrome B (mainly in green tissues), sensitive to red and FR light (660 and 730 nm, respectively) with reversibility, and sensitive to very high irradiance of FR, and phytochrome A (mainly in de-etiolation processes) sensitive to a wide range of wavelengths including FR (very low fluence rate reaction) and red-FR reversible reaction (germination) (Whitelam, 1995; Lin, 2000). Briggs and Olney (2001) have further shown that up to nine photoreceptors (five phytochromes, two cryptochromes, and one phototropin, plus one superchrome specific to ferns) are known in plants. Phototropins are important for chloroplasts movements, stomatal opening, leaf unrolling, etc. Studies with mutants have revealed complex interactions between these photoreceptors, interactions that buffer differences in spectral composition and intensity of light providing informational homeostasis (Mazzella and Casal, 2001). Unlike phytochromes, plants have various blue-light-UVA receptors that appear to be derived from more than one evolutionary lineage (Lin, 2000).

16.2. PHOTOSYNTHETIC RESPONSES TO LIGHT

16.2.1. The basics

Light responses of photosynthesis depend on conversion of photons into chemical energy, i.e., formation of reductive and energy equivalents NADPH and ATP, and use of

this energy to fix CO_2. Photochemical reactions are typically studied by Chl-F techniques, whereas gas-exchange methods are used to investigate CO_2 fixation. However, caution must be exerted with photosynthetic estimations from the former technique because the fluorescence signal is a mixture of contributions from different depths and layers within the mesophyll of the leaf (Evans, 2009; Oguchi *et al.*, 2011; see below on light gradients within the leaf).

During the photosynthetic process, sunlight is gathered by large arrays of light-harvesting pigment-protein complexes (LHC) and transferred to the reaction centres of PSI and PSII, where charge separation and stabilisation take place (see Chapter 2). Chl-F methods are based on re-emission of light as fluorescence from the red region of the spectrum (from 660 to 800 nm) owing to chl. *a*, mostly from PSII (see Chapter 10). Monitoring the yield of fluorescence makes it possible to estimate the fractions of absorbed light energy used for photosynthesis (photochemistry) and dissipated thermally as heat. There is always a balance between three possible routes for the absorbed light energy: photochemistry, heat dissipation and Chl-F. These three processes must cover 100% of the energy absorbed by the LHC of the photosynthetic apparatus.

In a fully functional photosynthetic system under low light (in dark-adapted state), most of the absorbed light energy is used for photochemistry, and only a minor fraction is thermally dissipated or re-emitted as fluorescence (Papageorgiou, 1975). However, all leaves have a certain finite capacity for photochemistry. As PPFD increases, and increasingly more PSII centres become closed, the fraction of light energy dissipated as heat and re-radiated as fluorescence increases. Under intense sunlight, more than 50% of the absorbed irradiance can be thermally dissipated (see Section 16.4 on excessive light and photoprotection). When the capability of the photosynthetic apparatus to convert the absorbed light energy photochemically is further impaired by any stress factor, the fraction of energy that is thermally dissipated can further increase to more than 90% (Demmig-Adams and Adams, 2006).

Over the past decades, the study of several parameters of the Chl-F emission together with gas-exchange measurements has become a rapid, sensitive and non-destructive method to investigate numerous aspects of the photosynthetic functioning (see Chapter 10), including the estimation of the photosynthetic electron transport rate (Genty *et al.*, 1989; Krall and Edwards, 1992; Evans, 2009). One of the most classical approaches is the measurement of gas exchange (CO_2 fixation or O_2 evolution) and PET as a function of light intensity (Genty *et al.*, 1989; Morales *et al.*, 1991, 1998, 2006).

16.2.2. Electron transport and gas-exchange rates as a function of light

The leaf-photosynthesis rate increases curvilinearly with increasing PPFD exhibiting saturation at intermediate to high light intensities. This response can be most commonly described by a non-rectangular hyperbola (e.g., Leith and Reynolds, 1987) that has three characteristic regions (Fig. 16.2): the initial slope (maximum quantum yield) where photosynthesis increases linearly with light; the curvature region, where photosynthesis starts to saturate with light; and maximum rate (maximum CO_2-fixation or O_2-evolution rate), where photosynthesis reaches an apparent plateau (Fig. 16.2). Measurements are made at a relatively low and narrow PPFD range, when the aim is to determine quantum yield, or increasing PPFD stepwise up to full sunlight, when the aim is to get more complete information on the light response curve of photosynthesis.

The quantum yield of photosynthesis (ϕ_{CO2}) is a measure of the efficiency of the photosynthetic process expressed in moles of photons absorbed within the PAR region per mol of CO_2 fixed or O_2 evolved (Ehleringer and Pearcy, 1983). The maximum quantum yield is measured when photosynthesis is light-limited (Fig. 16.2). The maximum quantum yields are calculated by linear regression from the initial part of the net photosynthesis versus light response curve, typically using a PPFD range from 20–30 up to 100–120 μmol photons m^{-2} s^{-1} (Morales *et al.*, 1991; Fig. 16.2). Quantum yields measured on an incident-light basis (called apparent quantum yields) are further corrected for leaf absorptance. In herbaceous species with thin leaves, leaf absorptance is ca. 80–85% of the incident light (Björkman and Demmig, 1987), but can be more than 90% for thick-leaved sclerophylls and conifers (Mesarch *et al.*, 1999; Niinemets *et al.*, 2005a,b).

The theoretical value of maximum quantum yield under CO_2-saturated conditions (in the absence of photorespiration) is 0.125 mol mol^{-1}, implying that 8 moles of photons are required to reduce 1 mole of CO_2 (Bolton and Hall, 1991). In general, the maximum value is closer to 0.112 owing to cyclic photophosphorylation, and it does not apply to C_4 species owing to the higher energy requirements of their CO_2-concentrating mechanism (Long *et al.*, 1993). Given also leaf absorptances of PPFD of the order of 0.85,

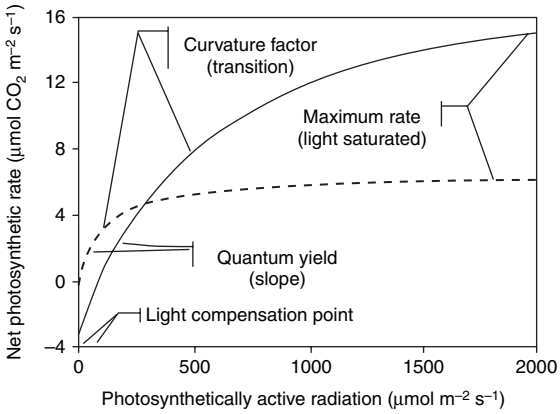

Fig. 16.2. Idealised net photosynthetic responses to photosynthetically active quantum-flux density (PPFD) of plant leaves acclimated to high light (continuous line) and low light (dashed line). Key parts of the hyperbolic relationship of photosynthesis are also shown: initial slope (quantum yield), curvature factor (transition from light-limited to light-saturated photosynthetic rate) and the asymptote (maximum photosynthetic rate). Light compensation point is the PPFD value at which net assimilation rate is zero (gross photosynthesis rate equals the respiration rate). Dark respiration rate is the rate of gas exchange at zero PPFD.

and other losses within the leaf (not all PPFD absorbed is absorbed by photosynthetic pigments), in practice maximum conversion efficiency of incident light is ca. 8–9% (Bolton and Hall, 1991; Ehleringer and Björkman, 1977; Ehleringer and Pearcy, 1983). In production-biology studies, the efficiencies are frequently calculated considering total solar radiation, which leads to efficiencies of ca. 1.8 times less, i.e., to maximum efficiency of ca. 4.5% for total solar radiation.

Quantum yields calculated from O_2 evolution might be higher than those calculated from CO_2 fixation because oxygen is evolved earlier and thus it is affected by fewer processes than CO_2 assimilation. Use of quantum yields calculated from CO_2 and methodological problems, such as including data extending beyond the linear region of the photosynthetic light response in quantum yield calculations, or changing levels of intercellular CO_2 concentrations during the measurements, can lead to underestimations of the maximum energy conversion efficiency. Strong correlations between the quantum yields and maximum potential PSII efficiencies, F_v/F_m ratios obtained by Chl-F in dark-adapted leaves, have often been found (Björkman and Demmig, 1987; Ögren, 1988; Demmig-Adams *et al.*, 1989). Measuring Chl-F to estimate maximum-potential PSII efficiency is less time consuming than measuring quantum yields, as a single shot of strong light of 1–2 s is required in the former, and generally at least six gas-exchange measurements at different light intensities (several minutes each) are required in the latter (Morales *et al.*, 1991). This is why in most ecophysiology laboratories Chl-F has replaced gas exchange as the default method to estimate maximum-potential photosynthetic efficiency (Adams *et al.*, 1990a).

Beyond initial slope, photosynthesis starts to saturate as soon as the photosynthesis in the first cell is light saturated. Owing to the light gradients within the leaves, mesophyll cells in different leaf layers are saturated at different quantum-flux densities (see Section 16.2.3) leading to the curvature of the light response curve of the leaf (Leverenz, 1988; Evans *et al.*, 1993; Ögren and Evans, 1993). In fact, there is often no apparent saturation of photosynthesis up to full sunlight in optically thick leaves, such as high-light-acclimated foliage (Fig. 16.2). For practical purposes, PPFD for 95% of theoretical maximum photosynthetic rate (estimated by fitting an empirical photosynthesis versus light-response-curve model to the data) is used as saturating irradiance in such cases (e.g., Niinemets and Tenhunen, 1997). Plants with aggregated foliage such as conifers constitute a challenge for measurements of photochemical yields and curvature of the light response. Owing to shading between foliage elements, awkwardly low quantum yields and curvature values can be obtained using standard measurement techniques suitable for broad-leaved species, commonly involving unilateral illumination with beam irradiance. For such species, correct estimates of photosynthetic light response can be obtained when the shoots are measured in the diffuse radiation field by placing the shoots into an integrated sphere (Öquist *et al.*, 1978; Leverenz, 1988).

Both gas-exchange and fluorescence methods can be used to study the responses of photosynthesis to light at curvature and saturating parts of the light response curve. Genty *et al.* (1989) found over a broad PPFD range a linear relationship between the PSII efficiency in light-adapted leaves, ϕ_{PSII} and the quantum efficiency of CO_2 assimilation under the same conditions, ϕ_{CO2}. This finding has been confirmed and extended by several authors for different plant species and for a variety of physiological conditions (see references in Morales *et al.*, 1998). However, different factors, such as water stress, SO_2 fumigation, cold stress and iron deficiency (Adams *et al.*, 1989, 1990a; Morales *et al.*, 1998), can decrease more ϕ_{CO2} (or ϕ_{O2}) than ϕ_{PSII}. Commercial portable instruments are available to measure Chl-F and CO_2

fixation simultaneously. They are tools to estimate photosynthetic electron transport rate (Krall and Edwards, 1992; see Evans, 2009 for an analysis of errors on assessing electron transport rate), quantify different electron-consuming processes (photosynthesis, respiration, photorespiration, etc.) (Valentini *et al.*, 1995a; Medrano *et al.*, 2002a) and identify any possible stress-mediated imbalance between electron generation and consumption (see Morales *et al.*, 2006, and references therein).

16.2.3. Light induction of photosynthesis

Upon illumination of a dark-adapted leaf, photosynthesis increases progressively for several minutes until reaching a steady state level, a process called photosynthetic induction. This process is important as photosynthetic responses to light depend on the degree of photosynthetic induction. Fully induced leaves respond immediately to any change in incident quantum-flux density, whereas induction significantly slows down the responses of photosynthesis to light, reducing the potential quantum-use efficiency. This plays a particularly important role in daily carbon gain in the understory, where leaves exposed to overall low diffuse irradiance sustain periods with high PPFD (lightflecks), and thus leaf photosynthetic apparatus undergoes repeated induction and deinduction cycles (Pearcy *et al.*, 1997).

During the induction, photosynthetic apparatus passes through different transitory stages. Electron transport reactions of photosynthesis become induced relatively rapidly. Their induction is reflected in changes in Chl-F yield (see Chapter 10) and lifetime (Morales *et al.*, 1999, 2001; Moise and Moya, 2004a,b). Upon sudden illumination of dark-adapted leaves by actinic light, Chl-F intensity increases up to six times (within a (few) second(s)) and then decreases to a stationary level (within a few min. after illumination). This transient Chl-F induction, known as 'Kautsky effect', reflects the PSII photochemical activity (Krause and Weis, 1991). The rapid kinetics of dark-adapted leaves has been shown to be an excellent tool to estimate the maximum potential PSII efficiency through F_v/F_m ratio (Morales *et al.*, 1991). Moise and Moya (2004a,b) have measured Chl-F yield and lifetime changes during the 'Kautsky effect' using phase fluorometry, and demonstrated that conformational changes in PSII and associated pigment-binding complexes occur during the dark-to-light transition.

The slower kinetics of electron transport, up to a few minutes, are reflected in reduction of Chl-F down to a steady state (F_s) level, owing to: (1) the partial re-oxidation of Q_A^- (photochemical quenching increases); and (2) the development of non-photochemical mechanisms (dissipation of excess excitation energy) (Buschmann, 1999). This is considered an essential regulatory mechanism of the photosynthetic control (Foyer *et al.*, 1990). Engagement of NPQ results in safe dissipation of absorbed light energy not used for photochemistry.

Simultaneously with commencing electron transport activities, Calvin-cycle enzymes, in particular Rubisco, ribulose-1-phosphate kinase etc. become activated (Leegood, 1990; Sassenrath-Cole *et al.*, 1994). Rubisco commonly exhibits the slowest rate of activation, and typically the Calvin cycle reaches full induction state within 5–10 min. after illumination (Leegood, 1990; Sassenrath-Cole *et al.*, 1994). The slowest process affecting photosynthetic light induction is commonly stomatal opening (Tinoco-Ojanguren and Pearcy, 1992, 1993a), where non-stressed plants take 5–10 min., but in water-stressed plants and under low humidity can take more than 30 min. (Tinoco-Ojanguren and Pearcy, 1993a; Aasamaa *et al.*, 2002). PET and carbon fixation are tightly coupled during photosynthetic induction, and low CO_2 concentrations owing to closed stomata reduce both the activity of PET (Ott *et al.*, 1999) (while enhancing energy dissipation) and Rubisco (Sage *et al.*, 2002). With the application of the saturation pulse method using modulated (PAM) fluorimeters (Ögren and Baker, 1985; Schreiber *et al.*, 1986), the relative importance of photochemistry and non-photochemical processes can be routinely determined at any time during the photosynthetic induction (see Chapter 10 for formulae and further explanations).

16.2.4. Gradients of light and photosynthesis within the leaf

Plant leaves have a complex three-dimensional architecture characterised by multiple cell layers consisting of cells with various size and shape, differently absorbing and scattering light within the leaves and collectively leading to complex within-leaf light gradients. Importance of leaf optical properties and features of the internal light microenvironment have often been discussed (Fukshansky, 1981; Vogelmann, 1989; Sun *et al.*, 1996; Nishio, 2000). Leaf internal architecture and distribution of pigments determine integrated leaf optical characteristics such as absorptance, reflectance and transmittance, but they can also importantly modify the responses of photosynthesis to light with differing intensity, spectral composition and directionality. Why leaves are green has been an intriguing question for a long time, and

the steep gradients of light within a leaf could provide a possible evolutionary explanation, because green light penetrates further into the leaf than red or blue light. By using chl.s that absorb green light weakly, a green leaf can achieve two conflicting requirements to maximise photosynthesis of the entire leaf: to increase radiation absorptance for photosynthesis and to maximise photosynthesis over all the chloroplasts of the leaf (Terashima *et al*., 2009).

In leaves lacking specialised anatomical features such as high waxiness, pubescence etc., total pigment content is the main determinant of bulk-leaf optical traits (Evans and Poorter, 2001). Nevertheless, leaf structure and anatomy influence the capture and internal processing of absorbed light (Vogelmann, 1989, 1993; Vogelmann *et al*., 1996). Depending on leaf optical properties and light spectral quality, the shape of the light gradients within a leaf can be relatively steep or gradual, exponential or linear. Leaf epidermal cells with thickened walls can act as lenses, focusing light into leaf-interior mesophyll cells (Poulson and Vogelmann, 1990). Sclereides and leaf bundle-sheath extensions can also act as optical fibres guiding light into the deeper interior layers (Karabourniotis *et al*., 1994; Nikolopoulos *et al*., 2002). In addition, long palisade cells can also canalise light into lower mesophyll layers enhancing photosynthesis (Vogelmann *et al*., 1996; Smith *et al*., 1997). Recent work has shown that at a leaf level, direct irradiance may have higher quantum efficiency than diffuse irradiance (Brodersen *et al*., 2008). This has been explained by canalisation of beam irradiance into the leaves by palisade cells (Brodersen *et al*., 2008; Brodersen and Vogelmann, 2010).

Thick leaves and those with a large proportion of spongy mesophyll with numerous intercellular air spaces, favour light scattering and promote light trapping. Owing to low absorptance of FR light (centered at 730 nm) by leaf pigments and leaf structures, the gradient in FR light is approximately linear, whereas blue light (centered at 450 nm) decreases exponentially with depth (Seyfried and Fukshansky, 1983; Vogelmann *et al*., 1989). Vogelmann *et al*. (1989) and Cui *et al*. (1991) have reported that blue light was largely attenuated by the initial 50–100 μm of leaf, consisting of the upper epidermal-cell layer and one-half to one palisade-cell layer. Green light is weakly absorbed, consistent with the absorption spectrum of chloroplasts, and penetrates deeper into the mesophyll than blue light (Evans, 1999). Red light strongly absorbed by leaf pigments creates steep gradients, although data reported to date indicate that red-light gradients are intermediate between those of blue and green (Evans, 1999; Evans and Vogelmann, 2006; Vogelmann and Han, 2000). In leaves irradiated with white artificial light or with sunlight, transmitted light is depleted in red and blue, and light scattered in the forward or backward directions consists predominantly of green and FR light. Thus, green and FR light dominate the light environment within the leaf both in the palisade and spongy mesophyll layers. These results indicate that blue and red light provide energy for photosynthesis in the cell layer(s) near the upper leaf surface, whereas green light is a particularly important light energy source deep within the leaf (Sun *et al*., 1998; Nishio, 2000). Although the chl. molar-absorption coefficient is small in the green spectral region, leaf absorptance is only moderately smaller (Morales *et al*., 1991). This is a result of the generally high leaf chl. concentration and to a larger effect of multiple scattering in spectral regions with weak absorption (see Louis *et al*., 2006, and references therein).

Cell composition and chemistry varies among different leaf layers. In *Spinacia oleracea*, Terashima and Inoue (1985a,b) and Terashima *et al*. (1986) have reported that chloroplast morphology and electron transport components gradually change from typical high-light-acclimated chloroplasts at upper mesophyll cells to low-light-acclimated chloroplasts in lower mesophyll cells (see Fig. 16.3). In paradermal leaf sections, chl. content per unit leaf layer reached a maximum at 75–105 ± 15 μm from the upper leaf surface (Vogelmann and Martin, 1993). Nishio *et al*. (1993) reported in paradermal leaf sections a gradual decrease in the chl. *a*/*b* ratio from the top to the bottom of the leaf. In addition, photosynthetic capacity also decreases from the upper- to the lower-leaf side (Evans *et al*., 1993; Ögren and Evans, 1993; Han *et al*., 1999; Evans and Vogelmann, 2003), but the gradients in light absorption and photosynthetic capacity do not necessarily match (Vogelmann and Evans, 2002; Evans and Vogelmann, 2003, 2006), implying that the leaf photosynthesis rate responds differently to light with varying spectral quality, resulting in changes in the curvature in the photosynthetic light response curve (Ögren and Evans, 1993). The mismatch between light absorption and photosynthetic capacity is especially pronounced for blue and red light that are already mostly absorbed by the upper-leaf layers, and is less for green light that is more uniformly distributed throughout the leaf (Evans and Vogelmann, 2003, 2006; Vogelmann and Evans, 2002). Such effects can be further amplified by CO_2 gradients within the hypostomatous leaves with stomata commonly on the lower-leaf surface (see Aalto and Juurola, 2002 for numerical simulation of within-leaf CO_2 gradients).

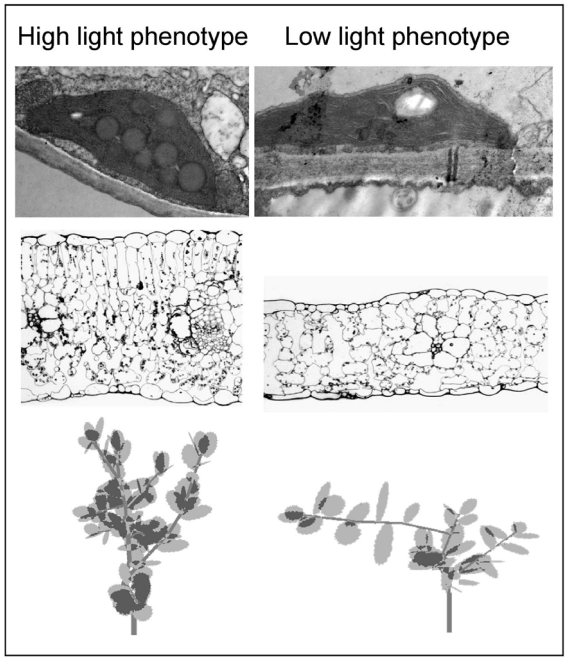

Fig. 16.3. Plant responses to light availability range from modifications at the level of organelles within the cells to modifications at whole-plant level. Phenotypes of a given plant growing in high light exhibit distinctive chloroplasts with highly stacked grana lamellae (upper images of transmission electron micrographs), thick, multilayered leaves (central images of light transmission micrographs) and dense crowns with steep branches and leaves (lower images of three-dimensional computer reconstructions of seedlings). Opposite suites of traits are observed in the same plant species grown under low light (right images).

Gradients in cell chemistry also have important implications for the photoinhibition sensitivity of upper and lower surfaces of bifacial leaves. Comparing the upper- and lower-leaf sides of *Phaseolus vulgaris* leaves, Louis *et al.* (2006) reported differences in chl.-excitation spectra, and ascribed them to a higher pool of carotenoids or to a greater conversion of violaxanthin to zeaxanthin on the upper side. Such gradients in photoprotection capacity along with gradients in photosynthetic capacity likely explain why the upper-leaf surface is more tolerant than the lower-leaf surface to high light exposure (Evans *et al.*, 1993; Sun *et al.*, 1996). Moreover, Oguchi *et al.* (2011) have recently shown that photoinhibiting leaves with lights of different colours results in different degrees of photoinhibition along leaf depth. Hence, although blue light induced the greatest photoinhibition, both near the adaxial surface and in the deeper tissue, red light induced greater photoinhibiton than green light near the adaxial surface but the opposite occurred in the deeper tissue.

16.3. PHOTOSYNTHESIS WHEN LIGHT IS SCARCE

Plant leaves growing in different light environments, ranging from more than 2000 μmol m^{-2} s^{-1} in full sunlight to less than 5 μmol m^{-2} s^{-1} in the forest understory, develop a set of morphological, physiological and biochemical characters improving photosynthesis in such challenging conditions. Under high light, plants optimise the use of light energy in photosynthesis (photosynthetic capacity) and energy dissipation, whereas under low light plants maximise light capture (Fig. 16.3). In this section, acclimation responses enhancing light-capture efficiency will be described with special reference to low light. As light is an extremely variable factor varying during the day, between days and during the season, plants growing in open places frequently encounter situations in which light is limiting for photosynthesis so its absorption must be guaranteed and kept sufficiently high to feed the high photosynthetic capacity. Also, there are always gaps in the canopy such that plants in the deep understory encounter bright lightflecks during the day and also demand photoprotection. A quantitative relationship between photoprotective energy dissipation (via the xanthophyll cycle, see below) and the characteristic growth light environments was shown across a large group of closely related species in the Hawaiian lobeliads, providing evidence for adaptive diversification in photosynthetic physiology (Montgomery *et al.*, 2008).

To obtain a positive carbon balance in low light, acclimated leaves display a set of morphological, biochemical and physiological adaptations (Valladares and Niinemets, 2008). Most of these traits are not easily reversible, and there are some 'obligate shade plants' for which low light is mandatory, often because of interacting stresses accompanying high-light environments. For instance, many bryophytes that lack advanced water-conducting elements and stomata are rapidly dried out under high irradiance (Proctor, 1984) and therefore prefer low-light environments. High-light acclimation of several 'shade plants' has also been shown to be limited by nutrient availability (Osmond, 1983). Nevertheless, most plants growing in the shade are 'shade tolerant' as they are able to develop leaves plastically and structurally, and are physiologically adapted to whole spectrum of irradiances (Valladares and Niinemets, 2008).

Morphological characteristics of low-light-acclimated leaves include thinner mesophyll with fewer or no layers of palisade parenchyma and fewer chloroplasts per area with larger grana and more stromal thylakoids (Fig. 16.3). Despite a decreased leaf mass per unit area and a low thickness, mesophyll conductance is often low in shade leaves, which contributes to a low photosynthesis capacity (Monti *et al.*, 2009). There are major differences in pigment composition between low-light and high-light-acclimated leaves in several respects: (1) chl. *a*/*b* ratio is commonly lower in low-light-acclimated leaves (e.g., Demmig-Adams, 1998) as a consequence of higher antenna size (LHCII enriched by chl. *b*) relative to reaction centers (PSI and PSII depleted from chl. *b*); (2) the ratios of photoprotective carotenoids to chl. are lower in low-light-acclimated leaves, reflecting lower demand for photoprotection. This is especially the case for the xanthophyll-cycle pigments, whose content relative to chl. can be fourfold lower than that in high-light leaves of the same species, indicating the increasing emphasis on light collection versus energy dissipation (Demmig-Adams, 1998; Niinemets *et al.*, 2003); and (3) presence in high amounts of some taxonomically restricted carotenoids. This is the case of α-carotene, lactucaxanthin (Demmig-Adams and Adams, 1996a), lutein 5-epoxide (García-Plazaola *et al.*, 2007) or *trans*-neoxanthin. In some species α-carotene may replace β-carotene, and lactucaxanthin may replace lutein in low-light versus high-light-acclimated leaves (Demmig-Adams, 1998). The presence of lutein epoxide increases the efficiency of light-energy use and simultaneously represents a reservoir for photoprotective lutein (Matsubara *et al.*, 2007) (see 16.5). In addition to these differences, some deep-shade tolerant plants also possess leaves with a reddish lower surface owing to the presence of a layer of red cells, characterised by high vacuolar anthocyanin content. This layer may serve to reflect red light, possibly increasing light scattering, penetrating the leaf again thereby favouring its re-absorption. However, the function of this red layer is still a matter of debate, for a deeper discussion see Chapter 7. Although the physiological meaning of all these modifications is still not completely understood, this evidence underscores the profound modifications in light-harvesting and the photoprotective-pigment system in acclimation to low light.

Apart from modification in pigment composition, acclimation to low light usually results in enhanced chl. content on a leaf dry mass basis ($chl._M$) (Niinemets, 2007; Hallik *et al.*, 2009). At the same time, thinner mesophyll is associated with lower LMA (Poorter *et al.*, 2009) allowing the plants to construct more foliar area with a given biomass in leaves. As the result of simultaneous modifications in LMA and $chl._M$, chl. content per leaf area ($LMA*chl._M$) that scales positively with leaf absorptance is generally weakly associated with light availability (Valladares *et al.*, 2002; Hallik *et al.*, 2009). Nevertheless, the light availability of single cells scales with leaf absorptance per unit leaf mass. Given that the absorptance per leaf mass scales with $chl._M$, leaf cells under low light do harvest light more efficiently than the leaves under high light (Evans and Poorter, 2001; Niinemets, 2007).

Biochemical differences between high-light and low-light-acclimated leaves reflect a trade-off in nitrogen allocation among light-harvesting and carbon-assimilating enzymes. Chloroplasts in low-light-acclimated leaves contain a larger proportion of antennae with fewer reaction centres, lower ATPase and lower Rubisco content, resulting in a greater fraction of nitrogen in LHC (Evans and Poorter, 2001; Eichelmann *et al.*, 2005; Niinemets, 2007). In low light, major antenna complexes of LHCII are dephosphorylated and aggregated with PSII core complexes, adjusting the energy partition between photosystems. Differences in nitrogen distribution among pigment-binding complexes and rate-limiting proteins of photosynthetic machinery, along with differences in LMA are responsible for the differences in photosynthetic-light responses between high-light and low-light-acclimated leaves: low-light-acclimated leaves have lower light compensation points, lower rates of dark respiration and photosynthetic capacity at saturating irradiance, but higher quantum yields for an incident PPFD (Björkman, 1981; Björkman and Demmig-Adams, 1994; Osmond *et al.*, 1999; Niinemets, 2007).

16.4. PHOTOSYNTHESIS WHEN LIGHT IS EXCESSIVE: PHOTOPROTECTION AND PHOTOINHIBITION

Photosynthetic tissues absorb light and transfer the excitation energy to the reaction centres under a wide and fluctuating range of PPFDs (Fig. 16.4). Photon absorption results in single-state excitation of chl. (^{1}Chl*), which may return to its ground state by transferring the energy to reaction centres resulting in charge separation and commencement of PET. Light capture and energy conversion must be efficiently coupled to the use of resulting chemical energy in photosynthesis. Whenever light-energy absorption by chl. surpasses its utilisation, the excess excitation energy can lead to generation of harmful ROS. In particular, high light intensities increase the risk of ROS production that

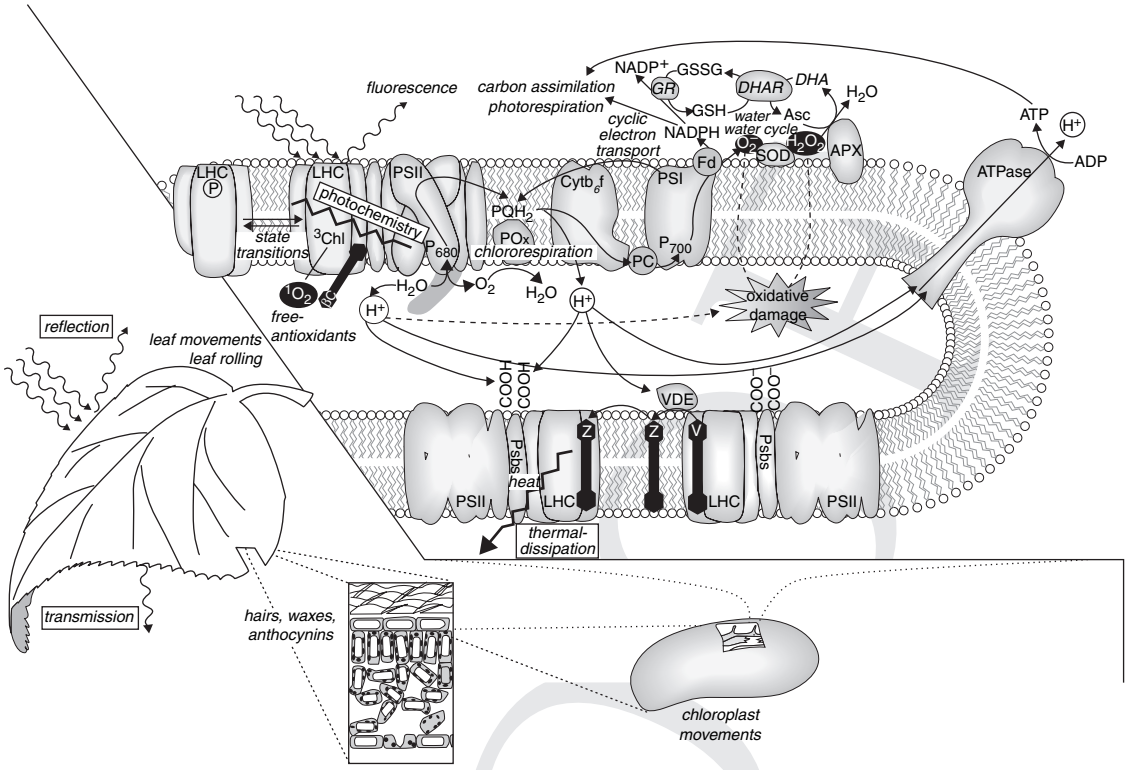

Fig. 16.4. Photoprotection mechanisms in photosynthetic tissues. When the photosynthetically active radiation (PAR) impinges on the leaf, only a fraction of it is reflected or transmitted. Morphological modifications, such as leaf rolling or movements together with chloroplast movements, and accumulation of reflective structures and compounds, such as hairs, waxes or anthocyanins, modulate the amount of light absorbed by chloroplast pigments. Redistribution of energy between both photosystems (state transitions), also contribute to reduce the excess of energy mostly under low-light conditions. Still the absorbed PAR might exceed the intensity that can be used in photosynthesis and a fraction must be dissipated as heat or canalised in alternative pathways to prevent damage to the photosystems. Thermal dissipation is modulated by the transthylakoid pH gradient and the operation of the xanthophyll (VAZ) cycle, whereas alternative energy sinks include the channelling of electrons through the water–water cycle, cyclic electron transport or chlororespiration. The last line of defence is constituted by the antioxidant metabolism and the action of repair mechanisms.

ultimately will result in generalised cellular damage, the so-called photodamage. Stress factors such as drought, high and low temperatures, air pollution, herbicides or mineral deficiencies reduce the rate of carbon assimilation, thereby exacerbating the risk of ROS formation. Photoprotection is achieved by a plethora of mechanisms: (1) structural avoidance of light interception; (2) enhanced metabolic usage of light energy; (3) conversion of absorbed energy into fluorescence or heat that is dissipated in the pigment bed; and (4) its conversion into ROS and subsequent scavenging. All these mechanisms act separately or simultaneously and at different time-scales ranging from seconds, in the case of heat dissipation, to long-term acclimation responses. They represent a trade-off between photosynthetic efficiency and photoprotection. In general, most morphological modifications to reduce light capture are considered as more static, often irreversible acclimation responses, whereas biochemical alterations constitute highly flexible and reversible acclimation to a fluctuating environment.

16.4.1. Avoidance of light capture

Leaves developed under high light absorb much more energy than they are able to use, and are acclimating to these high photon loads both biochemically and structurally. Biochemical avoidance of light capture is mainly associated with smaller antenna size and a higher carotenoid/chl. ratio, overall reducing light harvest at a given

incident quantum-flux density (Kitajima and Hogan, 2003; Niinemets, 2007; Takahashi and Badger, 2011).

Morphological avoidance of light interception is frequently the result of plastic and irreversible acclimation mechanisms (Fig. 16.3), serving to reduce the amount of light intercepted in leaves growing in open habitats. In general, high-light-exposed leaves of many species have steeper leaf angles in the top of the canopy to reduce interception of midday irradiances with highest intensity, whereas the leaves deeper in the canopy become more horizontal (Cescatti and Niinemets, 2004; Fleck *et al.*, 2003). Björkman and coworkers reported that such modifications in foliar angle can be reversible in herbaceous species, even at a timescale of hours or minutes (Björkman and Demmig-Adams, 1994). For instance, *Oxalis oregana*, an understory herb species adjusts leaf angle in seconds by turgor pressure changes in response to sunflecks (Björkman and Demmig-Adams, 1994). Other high-light-adapted species exhibit paraheliotropic (away from sun) diurnal or seasonal variation in leaf angles (Pastenes *et al.*, 2005), thereby significantly reducing light harvesting. Petiole curvature determining leaf movements is controlled by two processes, the induction and the cessation of curvature, and at least three photoreceptor systems are involved in the processes (Fujita *et al.*, 2008). In many species, high-light exposed leaves are more rolled with lower effective area for light interception (Fleck *et al.*, 2003). In herbaceous species, such rolling responses can occur relatively fast as the result of modification of turgor in bulliform cells in leaf epidermis, thereby reducing the light-harvesting surface when the potential excess irradiance load is the highest, for instance, during water stress, and increasing again the surface area under non-stressed conditions (Turgut and Kadioglu, 1998). In woody species with more rigid leaves that lack such specialised anatomical adjustments, leaf rolling is often irreversible.

At the sub-cellular level, chloroplast migration within photosynthetic cells represents the fastest morphological response to fluctuating PPFD. At low PPFDs chloroplasts in mesophyll cells are generally positioned side-by side perpendicular to the penetrating beam irradiance to maximise light interception. Upon exposure to high irradiance, chloroplasts move towards the vertical side walls of mesophyll cells, parallel to light direction. Chloroplast movements in plants are induced by blue light in a process mediated by a group of blue-light photoreceptors, the phototropins (Wada *et al.*, 2003). In thin leaves, chloroplast movements may reduce leaf absorptance by upto 20% in a few minutes (Björkman and Demmig-Adams, 1994), representing a cheap photoprotective strategy in fluctuating light environments. The adaptive value of chloroplast movement has been probed by the use of mutants defective in chloroplast avoidance (Kasahara *et al.*, 2002). However, in plants with optically thick leaves, chloroplast movement in upper mesophyll layers changes light-distribution gradients, but not necessarily much whole-leaf light absorptance.

Over time, many species regulate energy interception by changes in leaf reflectance. This is mainly achieved by the presence of trichomes, scales, air-filled spaces, salt crystals or epicuticular waxes (see Chapter 7). With a few exceptions, all these structures are spectrally neutral generating whitish (glaucous) leaves, which increase reflectance for the entire photosynthetic light spectrum. Through this process, leaves may reflect more than 50% of incident light, as occurs in the Mediterranean shrub *Encelia farinosa* (Björkman and Demmig-Adams, 1994). However, these modifications are slow or not reversible when light becomes limiting, and may become a disadvantage to plants under limiting light conditions.

An alternative mechanism to increase light reflectance is the accumulation of red pigments in the outer cell Reddening usually occurs transiently during leaf expansion or senescence but also under different environmental stresses (mineral deficiencies, drought, high or low temperatures or pathogens). Differently from structural features (e.g., hairiness) and compounds (epicuticular waxes) leading to glaucousiness, accumulation of red pigments is easily reversible, and disappears once the stress is over. However, there is still a controversy regarding their photoprotective functions, with two main hypotheses that are mutual but not exclusive: red pigments act as passive light filters or as antioxidants (Steyn *et al.*, 2002; Gould, 2004) (see Chapter 7). These pigments are mainly anthocyanins, but also betacyanins and carotenoids (Steyn *et al.*, 2002). These compounds increase red-light reflectance and absorb blue and green wavelengths, attenuating green light that can penetrate more deeply into the mesophyll.

16.4.2. Energy consumption by metabolism

Enhanced photosynthetic capacity allows for an increased use of photosynthetic energy. The main metabolic contribution to light-energy consumption ('dissipation') is CO_2 assimilation, whose potential capacity is commonly larger in leaves growing at higher light (see Niinemets, 2007 for a review). Photosynthetic capacity also increases in plants acclimated to sub-optimal temperature conditions

(Holladay *et al.*, 1992). However, under several other stresses limiting CO_2 entry into the chloroplasts, such as water stress, CO_2 fixation becomes heavily reduced and alternative metabolic processes gain importance. Other potentially major processes of metabolic energy dissipation are photorespiration (in C_3 plants; Kozaki and Takeba, 1996), nitrate reduction, the Mehler reaction (Osmond *et al.*, 1997), electron transport to ascorbate (Tóth *et al.*, 2009) and cyclic electron flow (Takahashi *et al.*, 2009). All these processes consume energy and reducing power, but their activities do not result in net carbon assimilation. They represent important energy sinks under conditions of restricted carbon assimilation. For instance, under water stress, photorespiration may consume the same amount (or even higher) of energy as that used in non-stressed conditions for carbon assimilation (Björkman and Demmig-Adams, 1994). The importance of photorespiration also increases with increasing temperature in parallel with the increased affinity of Rubisco for O_2. It is widely recognised that photorespiration protects C_3 plants from photooxidation, and new accounts for ecologically relevant situations of this protective feature are continuously reported (e.g., Zhang *et al.*, 2009d).

Other metabolic processes that enhance dissipation by acting as electron sinks are chlororespiration and cyclic electron transport (Bennoun, 2001; Joët *et al.*, 2002). The first represents a shortcut of the electron transport chain, in which a PQ oxidase oxidises $PQ_$, transferring electrons to O_2. The second consists in the CEFI or CEFII, with re-entry of electrons into the PQ pool or reduction of excited PSII ($P680^+$) by cyt b_{559}. Both mechanisms may act as a 'safety valve' to avoid over-reduction of PSII. In fact, *Nicotiana tabacum* mutants lacking chlororespiration were more sensitive to high light intensity (Peltier and Cournac, 2002). The mitochondrial AOX-respiratory pathway has been shown to protect the photosynthetic electron transport chain from the harmful effects of excess light, particularly under drought (Bartoli *et al.*, 2005).

16.4.3. Regulation of the efficiency of energy conversion

The efficiency of light-energy conversion into chemical energy can be finely regulated by chloroplasts (Fig. 16.4). The regulation of light energy conversion can be achieved by reorganisation of photosynthetic machinery by phosphorylation of thylakoid proteins. This process, called state transition, results in separation of LHCII and lateral migration towards PSI, and alterations in energy partition between PSI and PSII (Haldrup *et al.*, 2001). State transitions are important under low light, loosing importance under high light.

When excess light is absorbed, two additional main mechanisms reduce the yield of light-energy conversion: dissipation of energy as heat, which is proportional to the so-called NPQ, and the re-emission of photons as fluorescence. Although in terms of total energy, the part attributable to fluorescence is minor (ca. 3%), it is easily quantified and provides an essential tool for photosynthesis studies. NPQ is able to dissipate up to 99% of energy absorbed by chl. The non-photochemical energy dissipation mechanism is a rapid and flexible means for adjustment to the extremely variable light environment, switching from a highly efficient light-harvesting antenna to a low-efficiency dissipating system. *Arabidopsis* mutants lacking NPQ have demonstrated that this mechanism confers plants a strong fitness advantage as it increases plant tolerance to variations in light intensity (Külheim *et al.*, 2002).

Development of NPQ requires the build up of ΔpH across thylakoid membranes together with the presence of PsbS protein (Li *et al.*, 2000). Consequently, rates of NPQ increase whenever products of electron transport are not consumed in metabolic processes. Dissipation of excess energy as heat in the antenna is stabilised and amplified by the operation of the xanthophyll (or VAZ) cycle. In this cycle zeaxanthin is produced by light-induced de-epoxidation of violaxanthin, through the intermediate antheraxanthin, by the enzyme V de-epoxidase (VDE) (Müller *et al.*, 2001; Demmig-Adams and Adams, 2006). This reaction is controlled by the acidification of the thylakoid lumen (ΔpH) that occurs when chloroplasts are illuminated. Epoxidation back to V occurs in the dark or under decreased light pressure when the proton gradient is dissipated, completing the VAZ cycle and returning the leaf to a non-dissipating condition. Apart from the ubiquitous VAZ cycle, a parallel cycle that involves the light-induced de-epoxidation of lutein epoxide (Lx) to form lutein (L) has been described in some taxa of higher plants (García-Plazaola *et al.*, 2007; Förster *et al.*, 2009). The exact biophysical mode by which NPQ takes place is not completely understood, and several models have been proposed. Among others, the following mechanisms have been proposed: energy transfer from chl. to a low-energy carotenoid's excited state in LHCII (Ruban *et al.*, 2007), the charge transfer from chls to a chl.-Z

heterodimer in minor antenna components (Ahn *et al.*, 2008) or the direct dissipation within the PSII core complex (Finazzi *et al.*, 2004). These mechanisms are not mutually exclusive and all of them may operate simultaneously.

In addition to the ubiquitous mechanism of flexible NPQ associated with PsbS protein of PSII and controlled by ΔpH, under unfavourable conditions (specifically under low temperature) some perennial evergreens develop a process of sustained thermal-energy dissipation that does not relax with the darkening of leaves and disappearance of trans-thylakoid ΔpH (Demmig-Adams and Adams, 2006). Differently from ΔpH-dependent NPQ, sustained NPQ is neither flexible nor reversible. This mechanism is associated with PSII core-protein reorganisation and/or degradation and is characterised by a great increase in PsbS level, absence of ΔpH control, partial loss of D1 protein units from PSII core, a decrease in total chl. and the retention of high concentrations of de-epoxidised xanthophylls (A and Z) (Öquist and Huner, 2003). This mechanism is typically found under severe-stress conditions, such as those encountered by temperate evergreens in winter, leading to a sustained decrease of photosynthetic rates, the so-called 'winter photoinhibition' that covers the whole winter period.

Apart from their participation in energy dissipation, other mechanisms relating A and Z with photoprotection have been proposed, such as membrane stabilisation (Havaux, 1998) and antioxidant activity (Havaux and Niyogi, 1999; Havaux *et al.*, 2007). *Arabidopsis* plants overexpressing the gene responsible for Z biosynthesis (β-carotene hydroxylase) were also more tolerant to high light owing to the function of Z in prevention of oxidative damage of membranes (Davison *et al.*, 2002).

16.4.4. Detoxification of ROS

Sustained excess light can overwhelm the capacity for photoprotection, with the subsequent over-excitation of photosynthetic apparatus. This can lead to over-reduction of the electron transport chain and the conversion of singlet excited chl. (^{1}Chl*) into long-lived triplet chl. (^{3}Chl; Fig. 16.4). These effects ultimately result in the production of harmful ROS: singlet oxygen ($^1O_2$), the major ROS involved in photo-oxidative damage to plants (Triantaphylidès *et al.*, 2008), superoxide (O_2–), hydroxyl radical (·OH) and hydrogen peroxide (H_2O_2). The production of $^1O_2$ occurs mainly in the antenna by energy transfer from ^{3}Chl, whereas O_2– is primarily produced by direct oxygen reduction by PSI when the pool of NADP is mostly reduced (Mittler, 2002). The origin of H_2O_2 is photorespiration and the spontaneous or SOD catalysed dismutation of the O_2–. Some free metal ions in excess, such as Fe and Cu, and the Fe-S centre in PSI, may catalyse the production of ·OH by H_2O_2 reduction. A Fenton reaction may also occur between reduced Fe-S centres and H_2O_2. This reaction seems to trigger the photoinhibition of PSI (Sonoike, 1996).

If not chemically quenched, ROS can damage cellular components by direct oxidation of molecules. This is the case of the generation of peroxidation chains of polyunsaturated lipids. Even under normal growing conditions, ROS are produced as an unavoidable result of aerobic conditions, but any condition leading to a reduction in metabolic activity also enhances ROS generation (Osmond *et al.*, 1997). This process may threaten cell integrity, however, it also triggers acclimation responses, because ROS also participate in signalling events implicated in the process of acclimation to high light intensity (Mullineaux and Karpinski, 2002). In addition, it has been suggested that the degree of oxidation of antioxidant pools (e.g., ascorbate/dehydroascorbate) or elements of the electron transport (PQ pool) chain can serve as signals (Mittler, 2002). Thus, the role of antioxidant mechanisms is not only ROS detoxification, but also modulation of such stress signals, with photosynthesis playing a dual role in energy conversion and in the perception of environmental information.

To avoid damage, direct detoxification of ROS is carried out by a set of enzymatic mechanisms (SOD, CAT and ascorbate-glutathione pathway), which constitute the so-called water-water cycle, called this because the overall reaction implies water oxidation by PSII and water generation from ROS reduction (Asada, 1999). Photorespiratory H_2O_2 is deactivated by the enzyme CAT, whereas chloroplastic H_2O_2 is reduced to water by APX through the oxidation of ascorbate. This compound can be directly regenerated by monodehydroascorbate reductase (MDHAR) with NADPH as electron donor, or by the DHAR using GSH as electron donor. GSSG is again reduced with NADPH by GR. Active oxygen species can also be removed by several antioxidant molecules, either lipophilic (tocopherols and β-carotene, which can quench free radicals in membranes) or hydrophilic (ascorbate and glutathione). β-carotene is an efficient quencher of $^1O_2$ present in the core complex of PSII and in thylakoid membranes. Tocopherols also protect thylakoid membranes against ROS, and are able to terminate lipid-peroxidation chains. In high-light exposed leaves, the pools

of tocopherol are several-fold higher than in low-light-exposed leaves, suggesting that a higher capacity for membrane protection is needed at higher light (García-Plazaola *et al.*, 2004). Ascorbate is also an effective scavenger for peroxy radicals and $^1O_2$, which is able to act in synergy with tocopherols and is the electron donor for reactions catalysed by APX and VDE. Glutathione participates in direct or enzymatic detoxification of ROS, and acts indirectly as an antioxidant in its involvement in metal sequestration. Both antioxidants and enzymatic defense systems typically increase under stress conditions, but the accumulation of these molecules varied greatly with plant species and environmental conditions. Moreover, compelling evidence indicates that the set of physiological antioxidant molecules is much broader than previously thought, with many non-ubiquitous compounds being relevant in photoprotection, as is the case of hydrophilic flavonoids and lipophilic isoprene, monoterpenes and diterpenes (Peñuelas and Munné-Bosch, 2005).

16.4.5. Repair mechanisms

Repair mechanisms, such as the rapid replacement of photodamaged D1 protein in PSII core by newly synthesised units, represent the last line of plant defense against photo-oxidative damage. This protein is preferably damaged owing to the oxidation–reduction reactions that occur in the D1/D2 complex. The rate of photodamage of D1 protein depends on the redox state of the electron transport chain (Melis, 1999). Rapid D1 turnover in chloroplasts (60 minutes on average) contributes to avoid photodamage although D1 is in constant turnover. This process includes not only the synthesis of new D1, but also the complex disassembly of PSII complexes and removal of damaged D1, followed by reinsertion of newly synthesised D1 in the thylakoids and the reconstitution of the multi-protein reaction centers. Photodamage of D1 is a continuous process, but its rate increases linearly with irradiance (Takahashi and Murata, 2008) and with the size of the antenna (Melis, 1999). Retardation of the recovery of photoinactivated PSII can be a result of restricted mobility of PSII in the thylakoid membrane (Oguchi *et al.*, 2008).

Adverse conditions such as low or high temperatures also impair energy balance accentuating photodamage to D1, but recent studies also suggest that these stresses inhibit the repair of PSII by downregulation of the D1 synthesis (Takahashi and Murata, 2008). The balance between the rate of D1 damage relative to the rate of D1 repair finally determines whether sustained photodamage does occur in the leaves (Yokthongwattana and Melis, 2006).

16.5. SHORT-TERM VERSUS LONG-TERM RESPONSES TO RADIATION INTENSITY

Plants exhibit a wide range of phenotypic differences when growing in high versus low light (Fig. 16.3). Although some of these changes are slow and mostly irreversible, such as those involving the development of tissues and organs, other are relatively quick and reversible, such as those involving photosynthetic pigments and enzymes. Although all these changes reflect different aspects of phenotypic plasticity (i.e., the capacity of a given genotype to render different phenotypes under different environments), the former are classically included within the notion of plasticity (typically, developmental plasticity) and the latter are referred to as acclimation responses (Valladares and Niinemets, 2008). Whereas plasticity has been shown to increase light capture and photosynthetic utilisation (e.g., Valladares and Pearcy, 1998), not all plants exhibit the same levels of plasticity because there are many limits to and costs of a highly plastic response to a changing environment (Valladares *et al.*, 2007).

Leaf morphological and physiological adaptations to high and low irradiances received intense attention during the 1970s and 1980s, which led to a thorough description of the so-called sun and shade types of leaf (Björkman, 1981). Of course, light is a continuous variable, and leaf structural and physiological responses to light availability are also continuous (Niinemets, 2007), such that there is a spectrum of foliage structural and physiological variation between high- ('sun') and low- ('shade') light availabilities. This acclimation of foliages to their light environment is an important part of the potential phenotypic plasticity that plants can carry out in response to light availability (Valladares *et al.*, 2000a,b, 2002).

Leaves can acclimate to their light environment at several levels. For instance, they can modulate the leaf area per unit biomass and they can change the relative distribution of nitrogen between photosynthetic components (Evans and Poorter, 2001; Niinemets and Tenhunen, 1997). It was widely accepted that one of the most important differences between high- and low-light-acclimated leaves is the much lower respiration rates of the latter (Björkman, 1981). However, many studies have shown transient transfers to low or high light quickly reverse the patterns (e.g., Pearcy and Sims, 1994), and it seems that it is total daily carbon gain that drives

respiration rates more than acclimation. Besides, comparisons can be misleading as respiration occurs in response to growth and maintenance processes, which are restrained by light in the shade (Zaragoza-Castells *et al.*, 2007). The low respiration rates observed in the shade mostly reflect the low resource supply, but whether the capacity for respiration also adjusts is less certain (Pearcy, 2007). Another clearly established difference between high- and low-light-acclimated leaves is the leaf mass per unit area, which is larger in the former than in the latter. Leaves developed in the shade are generally thin, with a loosely organised mesophyll (Fig. 16.3; Niinemets, 2007; Valladares and Pearcy, 1999). The large differences in photosynthetic capacities per unit area of high- and low-light-acclimated leaves are reduced or absent when photosynthetic rates are expressed on a leaf-mass basis (Pearcy, 2007). This is consistent with a rather constant amount of photosynthetic machinery per unit leaf mass, and with a greater leaf mass per unit area of high-light versus low-light leaves. As leaf turnover is faster in high light, leaves developed under different PPFD also differ in their mean longevity; this has been related to the fact that the payback of foliage-construction costs under low light takes longer than under high light (Williams *et al.*, 1989).

Comparisons across many species reveal no consistent differences in leaf chl. concentration per A_{leaf} among leaves in contrasting light environments (Björkman, 1981). Average chl. concentrations per A_{leaf} are sufficient to absorb 80–85% of the incident PAR (Agustí *et al.*, 1994), the remainder being lost by either reflection or transmission. Although leaves in the shade could benefit from an enhanced light capture by an increased chl. concentration per unit mass, the costs are likely to exceed the benefits. A doubling of the chl. concentration is needed to increase leaf absorptance by just 5% from the 80–85% of absorptance of an average leaf (Niinemets, 2007; Pearcy, 2007). Although there are only 4 mol of nitrogen in a mol of chls, the associated proteins in chl.-protein complexes contain 21–79 mmol nitrogen per mol of chls (Evans, 1986). Thus, light harvesting by chl.-protein complexes involves an important fraction of the foliar nitrogen that may even be up to 60% of total leaf nitrogen (Niinemets and Tenhunen, 1997). The lower chl. *a*/*b* ratio observed in low-light-acclimated leaves has a negligible impact per se on light harvesting and quantum yield, but it reflects the cost of light capture per unit nitrogen invested: LHCII, where many chl. *b* molecules are located, contains only 25 mmol N per mol of chl., whereas PSII core complex, where many chl. *a* molecules are located, contains 83 mmol N per mol of chl. (Evans, 1986; Pearcy, 2007). Sun leaves cannot minimise the investment in PSII and save nitrogen in this way because they need the high electron transport capacity of PSII. There are clear pressures for increasing construction costs in both high-light and low-light environments: high-light leaves can be expensive because of their high concentrations of proteins (primarily photosynthetic enzymes), whereas low-light leaves can be expensive because of their high concentrations of anti-herbivore compounds associated with their long lifespan (Chabot and Hicks, 1982). High-light-developed leaves are more costly to construct on a per unit area basis, largely because of their greater mass per unit area, but on a per unit mass basis differences are minor. In broad surveys of leaf-construction costs, specific differences are small and no clear trends among habitats or growth forms have been identified, suggesting that leaf mass per unit area is more important than construction costs in determining differences in the carbon balance (Villar and Merino, 2001). This lack of consistent pattern seems to be owing to the variety of trade-offs between investments in structural, biochemical and protective compounds in different leaves from a given environment.

Plant responses to available light involve a suite of traits scaling from the cell to the whole plant. Photosynthetic capacity is linked not only to maintenance respiration but also to leaf-area ratio and leaf mass per area among the most relevant features involved in light acclimation (Pearcy and Sims, 1994). Relative growth rate (RGR) of plants is differentially affected by these four traits, depending on the light environment to which the plant is acclimated (Fig. 16.5). Photosynthetic capacity has a large effect on growth in high but not in low light, whereas the reverse is true for respiration. By contrast, the light environment has little effect on the influence of leaf-area ratio on growth, but model simulations reveal close links between these key plant features (Pearcy and Sims, 1994).

16.6. SUNFLECKS: PHOTOSYNTHESIS IN HIGHLY DYNAMIC LIGHT ENVIRONMENTS

Changes in the light environment associated with successional changes are relatively slow and predictable, which allow individuals to anticipate and respond; and the same applies to both seasonal and diurnal variations. But other changes can take place more rapidly and in a less predictable

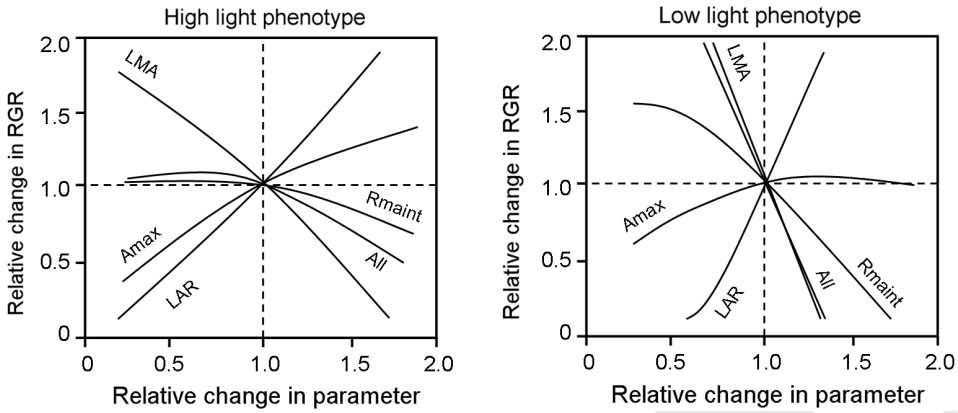

Fig. 16.5. Relative change in relative growth rate (RGR) in plants acclimated to high or low light as a function of a relative change in photosynthetic capacity (A_{max}), maintenance respiration (Rmaint), leaf area ratio (LAR), leaf mass per area (LMA) and all the four parameters according to a concerted acclimation response (all). The graphs represent a sensitivity analysis of RGR for the tropical plant *Alocasia macrorrhiza* modelled after experimental acclimation to contrasting light conditions. Adapted from Pearcy and Sims (1994).

manner (Fig. 16.6). Because the leaf area is heterogeneously distributed in the canopy, the resulting gaps allow penetration of sunflecks of various durations and intensities to lower canopy layers and the understory. Sunflecks cause the most rapid scale of temporal heterogeneity: typically from seconds to minutes. Sunflecks often contribute with a substantial fraction of the total light available in the understory (Chazdon, 1988; Pearcy, 1983). The constraints imposed by and the ability to respond to this fine-grain temporal heterogeneity are crucial to sunfleck utilisation and eventually to survival in dark understories (Pearcy *et al.*, 1994; Valladares *et al.*, 1997).

Most studies of the spatial heterogeneity of light focused on the variation of light regimes on large scales, comparing gaps with the understory or gaps of contrasting sizes, but a multilayered forest canopy may result in variations of light regimes on much smaller spatial scales (Canham *et al.*, 1994; Tang *et al.*, 1999). The size of the gap not only affects the duration of the sunfleck but also its intensity, because if it is not large enough (>0.5°) only radiation from a fraction of the solar disc will be received and the understory will be in partial shadow or penumbra. Most sunflecks in temperate and tropical forests are penumbral light, with maximum PAR intensity of 0.1 to 0.5 of full sunlight (Pearcy, 1990). As penumbral light is at a lower intensity than full sunlight, it can be used in photosynthesis more efficiently and can result in proportionally more carbon gain in the understory. The spatial scale of sunflecks typically vary from 0.1 to 1 m, so that often only part of the crown of an understory plant will be influenced by a given sunfleck (Baldocchi and Collineau, 1994).

Most of our knowledge on photosynthesis has been obtained from studies under steady light conditions, but light and photosynthesis are highly dynamic in nature. When light is suddenly increased during a sunfleck, photosynthesis will accelerate, but if the leaf has been in the shade for a long period, this initial increase can be small or almost missing because the stomatal opening is too small and because the enzymes of the photosynthetic carbon-reduction cycle are inactivated. Stomatal opening and activation of these enzymes is a relatively slow process, leading to the well-known induction requirement of photosynthesis in which the assimilation rate requires 10–30 minutes to reach its maximum value (Pearcy, 1988). If a fully induced leaf is shaded for a few minutes and then re-exposed to saturating light, only a few seconds are required for the assimilation rate to recover to its maximum value (Pearcy, 2007). Once the enzymes are activated, photosynthesis can continue in the shade at a high rate although for a very brief period of time in the so-called post-illumination carbon-fixation process. Post-illumination carbon fixation can contribute to as much as a 6% enhancement of carbon gain when very short sunflecks make an important contribution to the available light, as in the case of quaking aspen canopies under windy conditions (Pearcy, 2007). In the understory, induction limitations (stomatal limitations and Rubisco inactivation) predominate and significantly constrain carbon gain during sunflecks. Species differ in

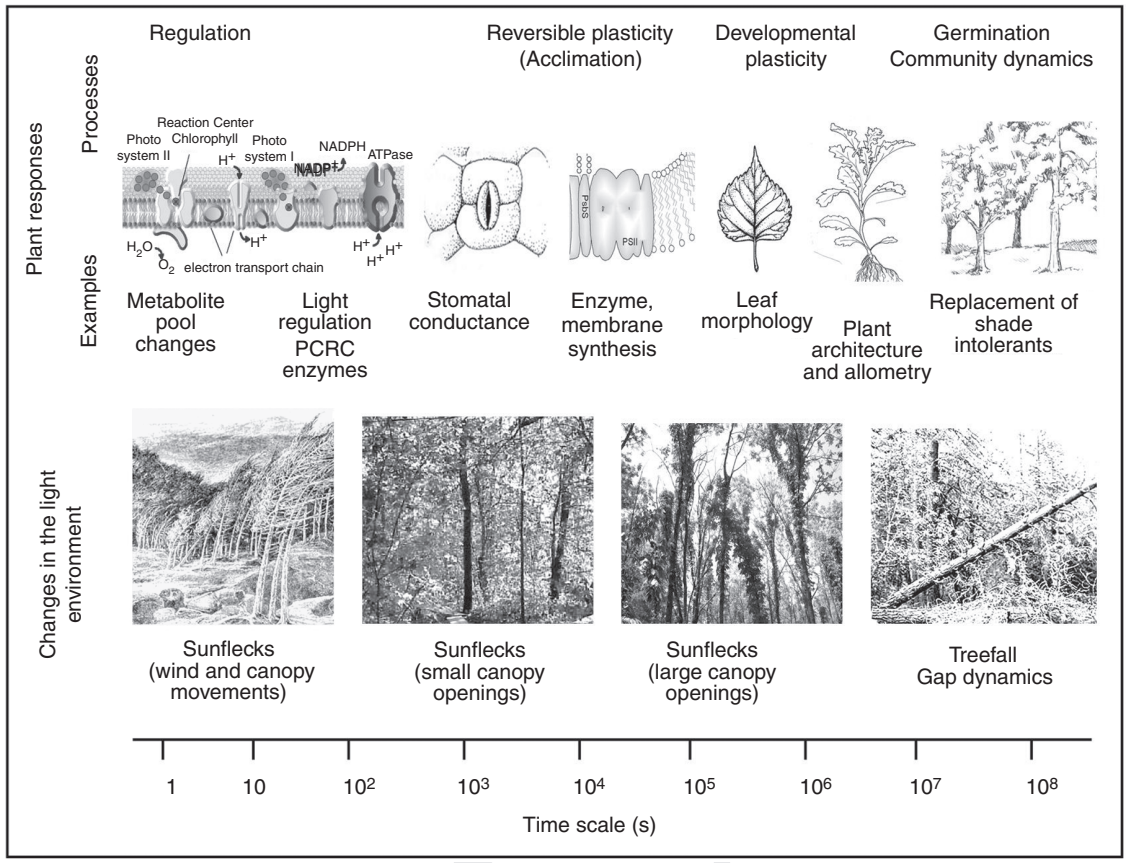

Fig. 16.6. Timescale from fractions of a second to years of changes in the light environment and in the responses of plants to these changes. Representative examples are given to illustrate both the sources of light temporal heterogeneity and the processes involved in plant responses.

their response to dynamic light and it has been shown that low-light species exhibit faster induction dynamics, remain induced longer and are more efficient in the photosynthetic use of sunflecks than high-light species (Valladares *et al.*, 1997; Pearcy, 2007).

16.7. GLOBAL CHANGE AND RADIATION

Climate change coupled with changes in land use and atmospheric features are inducing remarkable alterations of the light environment experienced by plants all over the world. They are leading either to an increased light availability (e.g., logging, clearing, fires, droughts) in certain areas, or to a decrease of available light in others. The latter is the case for certain temperate zones such as the Mediterranean, where land abandonment, reforestation programmes and strict conservation of habitats is reducing light heterogeneity and mean values of radiation available in the understory (Valladares, 2003). Whether many Mediterranean, typically high-light plants will be able to persist under this combination of global dimming and more extensive shade conditions is uncertain. Solar radiation reaching the Earth's surface has been significantly reduced by 0.51 W m^{-2} per year, equivalent to a reduction of 2.7% per decade (Stanhill and Cohen, 2001). The causes for this global dimming are unclear, but they seem to be primarily attributable to human-made aerosols and changes in cloudiness. And even less clear are the potential consequences of this reduction on ecosystem function in general, and on plant productivity in particular, especially in arid sites. Although productivity at high latitudes could be diminished by this reduction of solar radiation, in high-

radiation arid climates productivity could increase owing to reductions of water stress and photoinhibition (Stanhill and Cohen, 2001). The increased fraction of diffuse light associated with this global dimming is expected to increase productivity of complex plant crowns as transmission of diffuse light through the foliage is more efficient than that of direct light, but the issue is contentious and yet poorly understood (Roderick *et al.*, 2001; Gu *et al.*, 2002). Even the global trend on radiation is controversial because certain regions are experiencing a global 'brightening' (Wild *et al.*, 2005). Shade tolerance of the species and their photosynthetic utilisation of available light are inextricably involved in the responses of ecosystems to global change (see discussion in Valladares and Niinemets, 2008).

Many tropical and subtropical forests are experiencing an increased frequency and intensity of clearings and openings in the canopy. This perturbation is promoting the successful establishment of certain invasive plants, which seem to be remarkably plastic in their capacity to exploit the light available (Yamashita *et al.*, 2000; Durand and Goldstein, 2001). And the situation could be taking place in temperate forests as well. In a study of two contrasting populations of *Rhododendron ponticum,* an economically and ecologically relevant invasive species in many moist temperate areas, leaf-level plasticity in response to light was significantly higher than in *Ilex aquifolium,* a co-occurring evergreen shrub. A similar difference was also found between the two populations of *R. ponticum,* one autochthonous and the other one native and invasive: plasticity was linked to invasibility (Niinemets *et al.*, 2003).

Because photosynthetic (chls and carotenoids) and photoprotective (carotenoids and anthocyanins) pigments are positioned to capture solar energy they are well suited for detection from above (see Chapter 15). Using spectral reflectance, not only chls and carotenoids but also flavonoids, water, lignin, nitrogen-containing compounds and cellulose can be estimated by non-contact measurements of electromagnetic radiation (Gamon *et al.*, 1997; Gamon and Surfus, 1999). With the adequate ecological and physiological information for each particular case, remote sensing can be used to detect and quantify relevant biological processes in the canopy (productivity, water content and risk of fire, physiological status, radiation-use efficiency, chl. degradation), which is fostering research on ecosystem functioning at the landscape scale, a key issue to understand global-change impact on terrestrial ecosystems (Gamon *et al.*, 1997; Peñuelas and Filella, 1998).

The study of the biological effects of UV radiation, especially UV-B (280–315 nm), has attracted considerable attention during the last decades because the thinning of the stratospheric ozone layer leads to elevated solar UV-B at ground level (McKenzie *et al.*, 1999). Intense sunlight is associated with elevated UV-B, especially at high latitude or altitude. Field studies have shown that solar UV-B can reduce growth of certain species (Ballare *et al.*, 1996), but it can induce resistance against photoinhibition (Mendez *et al.*, 1999) or it can have few effects in other species (Papadopoulos *et al.*, 1999; Rousseaux *et al.*, 2001). Some of the most dramatic effects of solar UV-B have been observed in the Antarctic Peninsula with the grass *Deschampsia antarctica* (Day *et al.*, 1999).

Radiation is both a crucial resource and a heterogeneous factor, so even though plants depend on it, they more often than not must cope with either too much or too little energy. Global change is pushing the physiological, ecological and evolutionary limits of plants coping with light heterogeneity a bit further.