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Commentary

Safety conscious or living dangerously: what is the 'right' level of plant photoprotection for fitness and productivity?

Due to their sessile nature, plants could be perceived to be relatively slow and rather un-reactive. However, a plant scientist will tell you that the inability to run away (tropism notwithstanding) actually demands a highly sophisticated physiological response to the environment. Light presents an extreme case: cloud cover and wind-induced motion can lead to irradiance changes of several orders of magnitude over timescales of seconds and minutes. Being autotrophic organisms and having evolved to harvest light, plants need to dynamically regulate their biochemistry so that it operates efficiently during these fluxes, maintaining plant fitness but minimising the risk of damage.

Photosynthesis is driven at a rate that depends on the amount of available light, as shown by the schematic photosynthesis-light response curves of C3 species (Fig. 1). In nature, CO_2 assimilation can go from being light-limited to being light-saturated within a very short period of time. To maximise CO_2 uptake, photosynthesis should 'track' light levels accurately inducing and removing photoprotective processes accurately. Being able to measure photoprotection precisely in naturally fluctuating settings is difficult; however, a paper in this volume of Plant, Cell and Environment proposes a significant advance (Tietz *et al*. 2017).

Photosynthesis does not generally track changes in light level precisely (Pearcy & Way 2012; Lawson & Blatt 2014; Kromdijk *et al.* 2016). Why? The photosynthetic system is a complex and generally non-linear series of processes and reactions with many possible metabolic and physical limitations. On encountering high light, photosynthesis will remain low until light-dependent activation of enzymes, and other physiological processes take place: this includes activating the Calvin–Benson cycle, increasing metabolite pool sizes and opening stomata for CO_2 diffusion. At the same time, these processes are sensitive to changes in temperature, humidity and other factors. As a result, natural photosynthesis is often not at 'steady state'.

PHOTOPROTECTION VIA CONTROLLED ENERGY DISSIPATION (NPQ)

To add to the above, high light can be potentially deleterious. Light energy that is harvested by chlorophyll in the Light Harvesting Complexes (LHC) of photosystem II (PSII) is used by the reaction centres of PSII to split water via the oxygen evolving complex, using the resultant electrons and protons in the thylakoid membrane to generate ATP and reducing power for CO_2 assimilation. Chlorophyll is capable of absorbing light energy far in excess of photosynthetic requirements, which increases the likelihood of photoinhibition (light-induced reductions in quantum yield and possibly capacity) and oxidative stress. To an extent, plant cells are protected against this by photochemical activities themselves. The electron transport system is actually quite flexible with more than one photochemical 'sink', thus allowing electrons to be 'directed' in more than one way to avoid an over-reduced electron transport chain (Murchie & Niyogi 2011).

Photoprotection of photosynthesis describes a range of adaptations that help to prevent such over-reduction of photosynthesis and the onset of photoinhibition in high light. These can range from changes in leaf angle and chloroplast movement to inducible biochemical mechanisms. Of the latter, one of the most well studied is the controlled dissipation of excitation energy from chlorophyll which is measured as non-photochemical quenching or NPQ. This rather dull name belies an elegant and fascinating mechanism, ubiquitous among plants, that regulates the level of excitation within the pigment bed of the thylakoid membrane and partly determines the amount of excitation energy available for photosynthesis (Horton *et al.* 1996; Demmig-Adams *et al.* 2014). Photochemical and non-photochemical flexibility also allows the system to 'buffer' rapid changes in electron transport that could otherwise result in fluctuations and metabolic instability.

Under low light levels, with NPQ at zero, light energy absorbed by chlorophyll within LHCs is transferred efficiently to reaction centres via resonance transfer, and hence, the quantum yield of photosynthesis is at a maximum (Fig. 1). Higher light, especially levels that approach saturation of photosynthesis, results in the induction of the so-called high-energy state quenching (qE, which is often the dominant component of NPQ) and dissipates a portion of the chlorophyll excitation energy harmlessly as heat. Other components of NPQ include state transitions (qT; usually measured under low light) and inhibitory quenching (qI). qI can be formed from damage to reaction centres or other conditions resulting in sustained quenching and can be indistinguishable from photoinhibition (Horton *et al.* 1996; Belgio *et al.* 2014).

qE is induced rapidly (within seconds) and can rapidly relax (often within minutes) in comparison with qI. Chlorophyll excitation 'pressure' increases when absorbed light energy exceeds the capacity of the electron transport chain to use it and this is sensed via the electrochemical proton gradient across the thylakoid membrane. Thus, an increased proton gradient under high light results in qE formation. Major players in both the sensing and stimulation of qE include the protein PsbS and the xanthophyll cycle (XC). It seems that PsbS induces rapid formation and relaxation of qE, whilst

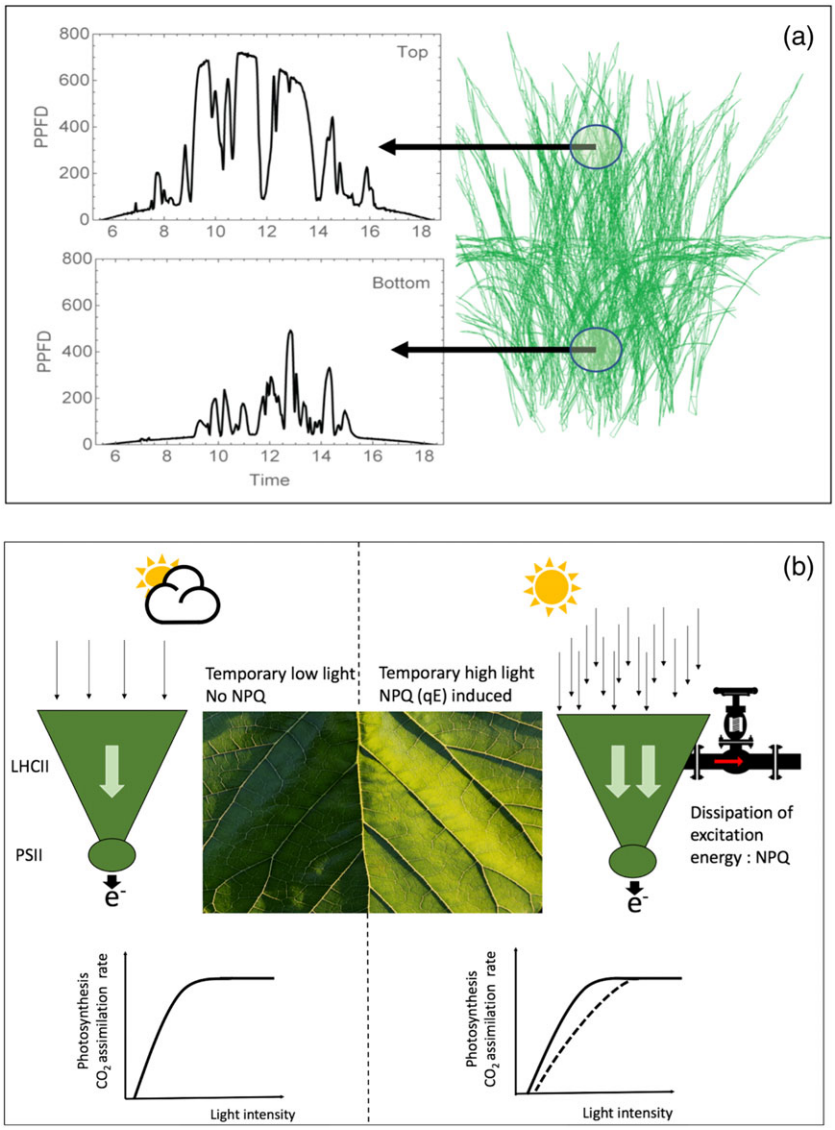

Figure 1. The complexity of the light environment and the relevance of thermal energy dissipation as measured by non-photochemical quenching. 1A shows fluctuations in light throughout the day in a sample part of the top half and bottom half of a rice canopy, here represented as a three-dimensional reconstruction. PPFD, photosynthetic photon flux density (image provided by Alexandra Burgess). 1B is a highly schematic figure intended to demonstrate how NPQ (here shown as high energy state quenching or qE) results in a momentary decline in photosynthetic efficiency in high light conditions. Light energy absorbed by the light harvesting complexes (LHCII) of photosystem II (PSII) is transferred or 'funnelled' to the reaction centre by resonance transfer mechanisms. Charge separation takes place in PSII and initiation of electron transport. In high light, qE results in thermal dissipation of excitation energy which reduces the likelihood of resonance transfer and the formation of long lived excited states of chlorophyll the latter being potential sites of oxidative stress. As shown here, qE can be thought of as a 'valve' dissipating excessive excitation energy from the pigment bed of the photosystem II complex (Demmig-Adams & Adams 2006). The outcome of this process for CO_2 assimilation is shown by the dashed line in the light response curves under high light. Should a leaf be transferred from high to low light, the momentary reduction in quantum yield will lower productivity.

XC-induced qE is slower to relax. These different dynamics confer a means of providing highly flexible protection to the photosynthetic apparatus over different timescales within unpredictable fluctuating environments (Demmig-Adams & Adams 2006). It has been suggested that one role of the XC is to give the plant a 'memory' of recent high light events allowing rapid protection again should it be needed (Murchie *et al.* 2009).

All forms of NPQ will, momentarily at least, lower the quantum yield of photosynthesis (the efficiency of CO_2 fixation under low light), thus conferring a conceivable 'cost' to photoprotection. The fact that the qE memory persists in low light in naturally fluctuating conditions has led to the suggestion that it can limit photosynthesis (Murchie *et al.* 2009; Kromdijk *et al.* 2016). This is important because the 'mismatch' between light levels and the photoprotective state of chloroplasts probably determines yield of plants and crops. Recent remarkable work which manipulated the dynamics of qE has shown that accelerating qE recovery in low light increases crop yield (Kromdijk *et al.* 2016). An important point

is that even though qE is protective, it is still not clear how much and when, is needed to avoid over-protection and to maintain high productivity.

SIGNIFICANT STEPS IN THE MEASUREMENT AND UNDERSTANDING OF PHOTOPROTECTION

Thermal energy dissipation (qE) is clearly a process with global significance, safely processing huge amounts of solar energy absorbed by terrestrial vegetation and algae and hence directly determining productivity, at least in some systems. It also has emerging roles in signalling diverse processes such as pathogen attack and herbivory. However, research in this area has been hampered by the fact that to accurately measure NPQ, one needs to dark-adapt leaves or plants for many minutes to fully relax qE (which may not always happen). This presents practical problems, both for studying NPQ in dynamic environments and for making routine high throughput measurements such as those that are used in modern phenotyping or imaging of entire leaves and canopies in the field.

In this issue of Plant, Cell and Environment, Tietz *et al.* describe a method for measuring NPQ which has the potential to overcome the issue of dark-adaptation and permits access to new possibilities for our understanding of dynamic photosynthesis and its application to crop improvement and plant productivity. NPQ is conveniently measured non-destructively in leaves using chlorophyll fluorescence (CF) which is the low-level re-emission of light by chlorophyll, the yield of which is closely related to both the photochemical and non-photochemical quenching of excitation energy.

NPQ calculation normally requires a dark-adapted value of the minimal and maximal CF yield, which is partly related to the fact that fluorometers measure a value proportional to CF yield rather than CF yield directly. The different properties of each sample and each leaf require dark-adapted values to be measured each time. The parameter $NPQ_{(T)}$, derived in full in Tietz *et al.* (2017) critically utilises the long-held observation that the maximum quantum yield in the absence of any NPQ (Fv/Fm) of leaves has been empirically determined as 0.83 (Björkman & Demmig 1987). Conceptually similar approaches have been used to develop methods for measuring photoinhibition and calculating the proportion of total NPQ that is effective in protecting NPQ (Ruban & Murchie 2012; Ware *et al.* 2015).

The upshot is that $NPQ_{(T)}$ can be measured in the light as rapidly as other CF parameters such as the quantum yield of photosystem II (ΦPSII) and without the need for prior dark-adaptation of the leaves. The advantages are substantial: dynamics of NPQ can be directly tracked over short timeframes in naturally fluctuating environments (Fig. 1) whereas previously this would usually have required proxies.

The authors utilise two pertinent examples of why this method overcomes old issues with NPQ. First, chloroplasts move over periods of minutes around palisade mesophyll cells according to available light, notably toward shaded walls when the leaves are exposed to high light. This upsets NPQ measurements via an underestimation of maximal fluorescence in the light. Tietz *et al.* show that $NPQ_{(T)}$ is not affected by such movement because of the brevity of the measurement. Second, they show the advantage of $NPQ_{(T)}$ in plant canopy imaging where leaf movement (e.g. tropism and nastic responses) again can upset NPQ measurements because they depend on knowing the position in which the original Fm measurements was made.

The authors rightly point out some caveats of this method including the accuracy of the empirically derived Fv/Fm maximum (0.83). Any deviation from 0.83 may not be caused by residual NPQ but perhaps antenna (LHC) detachment. In theory, the maximum value can be adjusted accordingly, and some knowledge of the plant material would be necessary for more precise in depth analyses. So it may still be necessary in some experiments to confirm Fv/Fm further via dark-adaptation. But overall, this approach lends itself especially well to modern and dynamic investigations and to plant phenotyping which uses a high density of measurements across wide spatial and temporal scales.

CONCLUSIONS

We are beginning to fully appreciate that plant productivity and yield is highly dependent on chloroplast thermal energy dissipation as measured by NPQ (Kromdijk *et al.* 2016). This is closely linked to dynamic responses during natural environmental fluctuations, and such properties are not necessarily predictable from common steady state measurements. To understand this somewhat stochastic field, we need to be able to directly measure photosynthesis and photoprotection during these dynamic changes, a difficult task. Another critical issue is being able to readily separate protective from non-essential quenching (Ware *et al.* 2015). From the above, it is clear that NPQ should now be an important inclusion in crop improvement programmes. Therefore, further development of NPQ measurement technology and its application in applied and biological settings is essential.

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CONFLICT OF INTEREST STATEMENT

I have no conflict of interest to declare.

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