Chapter 2

Photoinhibition: Then and Now

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Summary

This perspective advocates a holistic view of photoinhibition from the molecule to the biosphere; a view that integrates many biophysical and biochemical processes in antennae and reaction centers of the photosystems that, when acting in concert, allow plants to respond to diverse and dynamic light conditions in many different environments. We take the general view that photoinhibition refers to a reduction in the efficiency of light use in the photosynthetic apparatus (Kok, 1956). Since the 1970s, biochemical, ecophysiological, and genetic studies of photosynthetic functions in strong light, *in vivo* and *in situ*, and their interactions with biotic and abiotic stresses, have significantly advanced our understanding of photoinhibition. We trace some origins of the idea then, that slow dark reactions, such as growth, CO_2 assimilation, photorespiration, and photosynthetic electron transport, ultimately limit light use in photosynthesis, and thus determine whether light is in excess and the magnitude of "excitation pressure" in the photosynthetic apparatus at any moment. This and other ideas are followed through studies of photoacclimation in leaves of plants and algae from diverse terrestrial and marine environments.

We highlight two currently interesting possibilities for the photoprotective dissipation of "excitation pressure" that reduce the efficiency of photosynthesis by changes in structure and function of antenna pigment-protein complexes and in the populations of functional and non-functional PS II centers. We conclude by briefly considering challenges presented now by the discovery of "gain of function", very high light resistant (VHL^R) mutants of *Chlamydomonas*, by the accessory lutein-epoxide cycle, and by technologies for remote sensing of photoinhibition in the field.

I. What Then?

This perspective reviews some hypotheses that have stimulated research in photoinhibition since its renaissance in the 1970s through studies of the slow, dark reactions of photosynthesis, and their implications for the faster, primary light reactions. Then as now, hypotheses were only as good as the experiments they provoked, so these ideas flourished as tools improved for assessing photosynthetic functions in vivo and in situ. Our eclectic pursuit of these ideas would have been impossible, then or now, without generous access to the needed

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infrastructure, made available in a timely fashion by a network of collaborators worldwide.

In the 1970s the Research School of Biological Sciences (RSBS), Institute of Advanced Studies at the Australian National University provided a stimulating setting for creative colleagues and students of great initiative. Barry Osmond drifted into research on photoinhibition unfettered by peer review (that *"well-meaning but narrow-minded nanny of an institution* (*that*) *ensures that scientists work according to conventional wisdom and not as curiosity or inspiration moves them"*; Lovelock, 1990). This environment also attracted distinguished visiting scientists and foundations were laid then for many later collaborations and expansion of ideas in other laboratories as one's distractions and responsibilities multiplied. In one of these, a generation later, an exchange program brought Britta Förster from the Freie Universität Berlin to the joint laboratory of John Boynton and Nick Gillham at Duke University. There we were fortunate to find our ideas accepted in an established genetic program where a decade of support from an exceptionally broad-minded, peer-reviewed program of basic energy research in the US Department of Energy allowed us to pursue genetic analyses of key processes in photoinhibition.

Recalling that a picture is worth a thousand words, we would like to bracket this essay with two images created by the processes of photoinhibition. These images carry us from a century old, then comprehensive account of "assimilation inhibition" by strong light (photoinhibition), to visualization now of the questions of photoinactivation and photoprotection in chloroplast grana. Our images were printed by photoinhibiting leaves from a plant (*Cissus*) grown in deep shade, by exposure to full sunlight while covered with 35 mm black and white film (negative and positive; Osmond et al., 1999). Photoinhibition occurred in leaf cells under the more transparent areas of the film, quenching chlorophyll fluorescence so that a positive image could be revealed by a filtered digital camera system, or resolved to the level of granal stacks of chloroplasts in leaves using a confocal microscope.

Text excerpts of Ewart (1896) on a microfiche negative remind us of a century of progress in technologies available for photosynthetic research (Fig. 1). Ewart observed "assimilatory inhibition" (reasonably equated today with photoinhibition) in leaf tissue slices from shade plants after exposure to strong light. Using Engelmann's method, he found that fewer O_2-tactic bacteria congregated adjacent to chlorophyll bodies (chloroplasts) in leaf cells after strong light treatment, indicating reduced O_2 evolution (indicating photoinhibition). Although we have now come a long way technologically, many of the questions addressed by Ewart remain unresolved today. One may ask why so little attention was paid, for so long, to evidence exhaustively assembled by Ewart and many others, indicating exposure to "excess light" impairs the efficiency of photosynthesis?

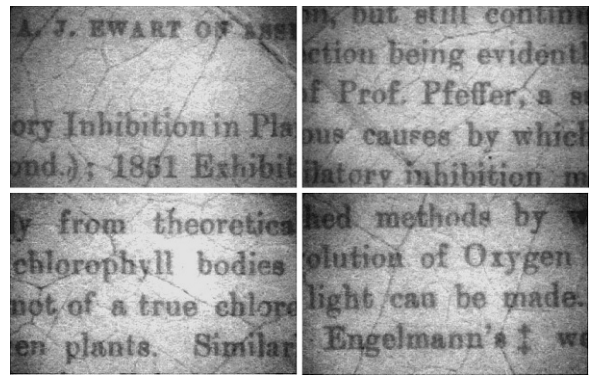

Fig. 1. Photoinhibitory print on a *Cissus* leaf, viewed as chlorophyll fluorescence, showing text excerpts from the introduction of Ewart (1896) that describe the context and methods of his early investigations of what we now refer to as photoinhibition (Osmond et al., 1999, with permission).

It may be that most of the chlorophyll on the planet is to be found in the shade and, by focusing on understanding the principles of efficient light utilization in weak light, we took it for granted that plants must have evolved sophisticated means of dealing with exposure to strong light. Negative and positive phototaxis is widespread among motile photosynthetic cyanobacteria and algae, but among immobile higher plants comparatively few have evolved light avoiding leaf or chloroplast movements (Kasahara et al., 2002). Some plants, like *Cotyledon obiculata*, manage external photoprotection with reflective wax and only engage internal, xanthophyll de-epoxidation defenses when brush-wielding, curiosity-driven researchers interfere with the natural order of things (Robinson and Osmond, 1995). Although shade and sun species, and even shade and sun ecotypes, can be distinguished, many plants survive and grow in habitats of glaring sunlight so there seemed to be no obvious problem. As tools emerged for assessing photosynthetic functions of plants in the natural environment, it became clear that photosynthetic efficiency declined in "excess light" and in response to stress. We now know that molecular mechanisms to repair photoinactivation or photodamage, and strategies for "lowering the shades" through photoprotection, have evolved to ensure photoinhibition in strong light is usually reversible. Although