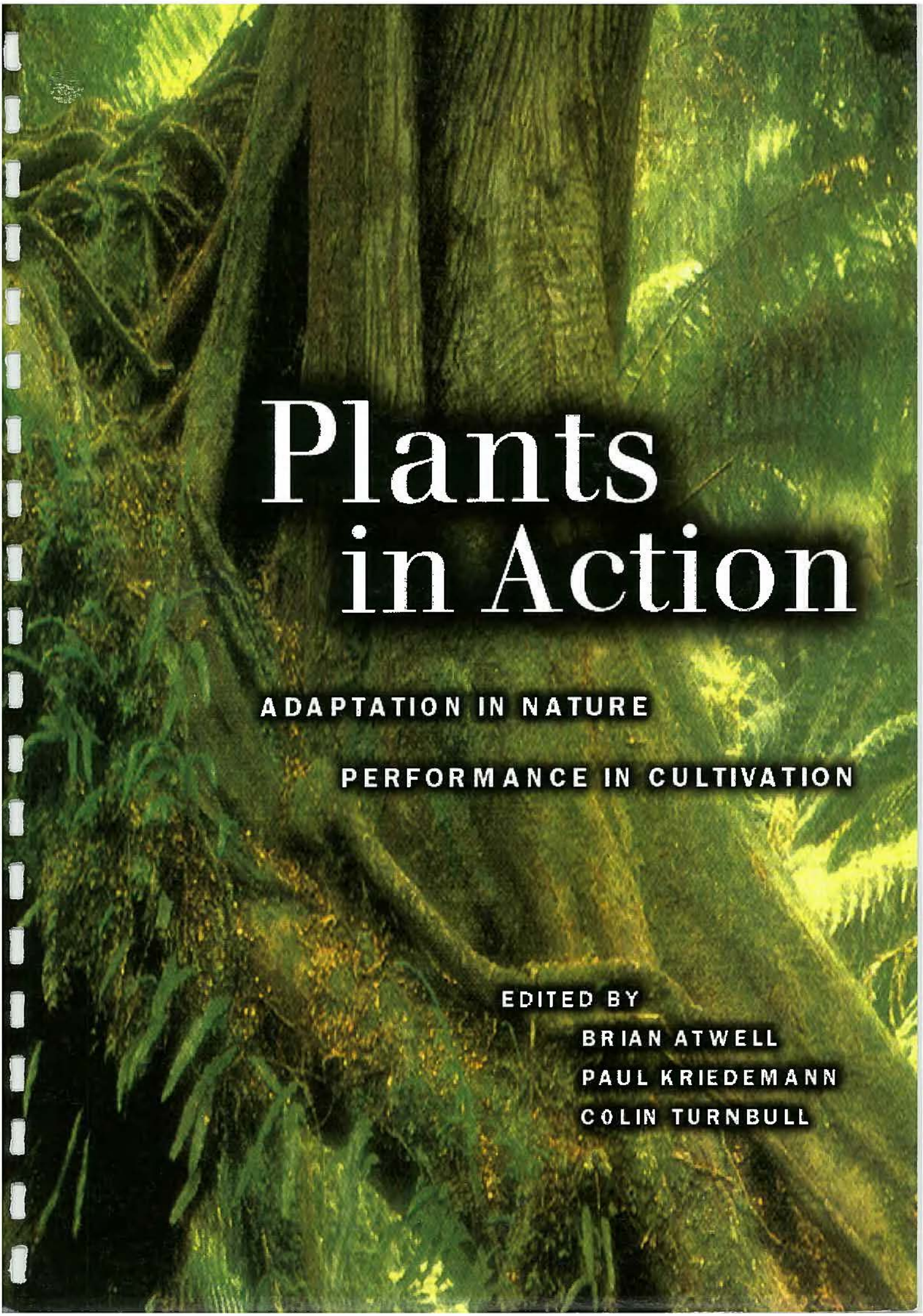
Plants
in Action
ADAPTATION IN NATURE
PERFORMANCE IN CULTIVATION
EDITED BY
BRIAN ATWELL
PAUL KRIEDEMANN
COLIN TURNBULL

Plants in Action

Dedicated to Emeritus Professor Sir Rutherford Robertson (affectionately known as Bob Rob), in recognition of his formative influence on plant science in Australasia

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Plants in Action

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(*Nymphaea violacea*) and grasses (*Pseudoraphis spinescans*), courtesy of Surrey
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Preface

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Part I

Perspectives on plant science

Part I Contents

Preamble

Ralph O. Slatyer
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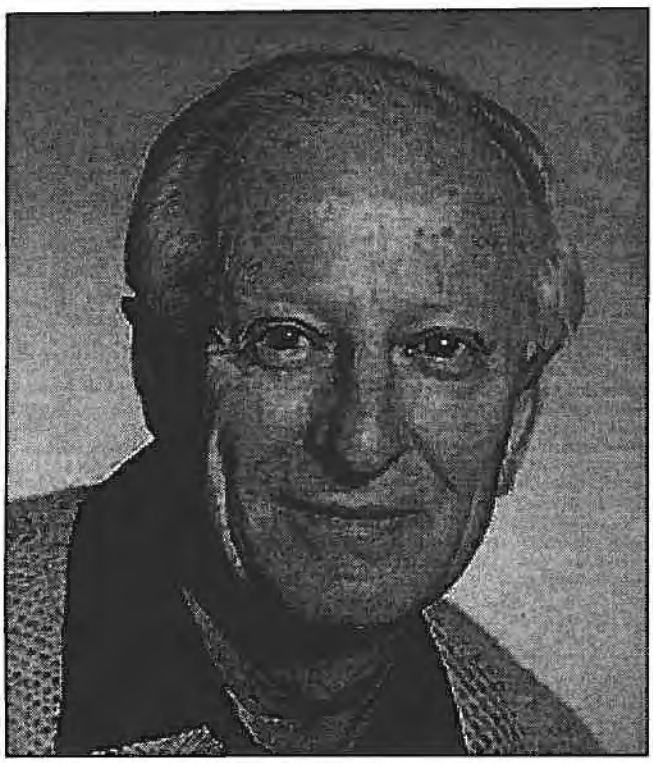


Figure I.1 Professor Ralph Slatyer, AC, FRS, FAA, FTSE

Green plants provide the biological energy for the functioning of the biosphere. All other organisms depend upon it for their livelihood.

Nowhere is this dependence more evident than with human beings since nearly half the products of global photosynthesis are channelled directly through human populations or are utilised indirectly by us for the myriad of activities that we undertake.

Our overall impact on the environment is the product of the total number of people and the average impact of each person. Continued growth in population, and in the demands of individual people, are not only increasing the overall impact but are resulting in a reduction in the area of arable land and in land degradation, thereby reducing the scope to provide for human needs.

In many regions of the world, particularly those already overpopulated in terms of rural productivity and the carrying capacity of their land, these factors are resulting in political, economic and social upheaval.

Clearly this increasing overall impact must be reined in. But for the next few decades at least, global population will inevitably continue to grow, by which time it may be double the present level. There is therefore a pressing need to reduce the impact per person and this applies more to agriculture than to many other human activities.

To provide for future generations agricultural ecosystems will have to be ecologically sustainable and the total sustainable production must be adequate for our increased numbers. It follows that sustainable yields from existing agricultural ecosystems will need to increase, and soils and climates now regarded as marginal or unsuitable for agricultural production may need to be brought into cultivation.

Climate change is an additional issue. On the positive side it may lead to more favourable climatic conditions and increased yields in some existing agricultural areas and may result in some areas now regarded as climatically marginal for agriculture being brought into cultivation. On the negative side it may result in the climate of some existing arable areas becoming marginal or unsuitable for agriculture. Furthermore, climate change may shift favourable climatic zones into areas of less fertile soils.

Conservation of biodiversity is an essential requirement for ensuring ecosystem function at a local, regional and global level. Biodiversity is threatened by habitat destruction and

modification, and by fragmentation of areas of natural habitat. Gene pool erosion and species extinction can occur as a direct result of these changes and indirectly as fragmentation and isolation of previously intact areas reduces habitat requirements below the minimum needed for population and specie viability.

Climate change exacerbates this situation since habitat modification and fragmentation hinder, and may prevent, natural migrations which have been a primary mechanism by which species have adapted to altered environments and gene pools have evolved.

These complex issues present a major challenge to plant scientists, particularly since the solutions must endure not just for one or two generations but indefinitely if human beings are to live on earth in peace and harmony.

The title and subtitle of this book, Plants in Action: Adaptation in Nature and Performance in Cultivation, succinctly summarise the challenge to plant scientists for the future. For plant scientists to respond to this challenge and to play a role in improved rural production and biological conservation, we need to understand better the mechanisms by which changes in the physical environment affect physiological processes, the manner in which genetic control over physiological processes is exercised, and the means by which genomes can be modified to produce cultivars which can be successful in modified environments.

And for ecological sustainability, it will also be necessary to understand better the mechanisms by which changes in the physical environment affect the interactions between species and determine the range and limits of species distribution.

There is a wealth of genetic material in the world's biodiversity to draw upon in modifying the genomes of existing and future cultivars to provide a basis for better adapted cultivars and sustainable agricultural ecosystems in climates and on soils at present regarded as unsuitable for agriculture. There is also the prospect of modifying the genomes of native species with a view to ameliorating the effects of fragmentation and degraded habitats on gene pool erosion.

Can the plant science community meet these challenges? There is clearly much to be done and much to be learned, but already there have been substantial achievements. Among these are the success stories in nutritional physiology associated with the discovery of trace elements. Research into salinity tolerance and the development of forest and crop cultivars which can tolerate saline substrates has shown the potential for addressing what were thought to be intractable environmental conditions.

There also continue to be major advances in the portability and accuracy of instruments used for physiological research so that experiments and observations previously restricted to laboratories can now be conducted directly in the field. This is enriching the whole field of ecophysiology and is opening the way for physiological information to be applied more directly to agricultural and ecological problems.

On the other hand, major challenges remain. Much remains to be learned about morphogenesis and the effect of water and heat stress and enhanced CO_2 levels on plant growth and development. The control of floral initiation remains elusive yet has enormous consequences for the development of cultivars with different times to maturity. Such developments would open the possibility of multiple cropping in favourable environments with obvious implications for yield improvement.

Stomatal function is a vital factor in mediating the response of plants to water stress and to high CO_2 environments and offering scope for enhanced water use efficiency. Yet our present understanding of stomatal function is not adequate to explore the possibilities of utilising genetic variation as a tool in modifying the stomatal characteristics of cultivars. Even our understanding of the effect of environmental factors on photosynthesis and respiration, probably the most studied and best understood of all physiological processes, requires strengthening.

This book will be of particular value to students, and to the plant science community generally, because gene–environment interaction is a pervasive theme and it gives special emphasis to plant responses to environmental conditions and to stress environments generally. Its Australasian roots will ensure that it is relevant not just to the problems of developed countries but also to those of developing countries.

A plant science manifesto

John Passioura

CSIRO, Division of Plant Industry, Canberra

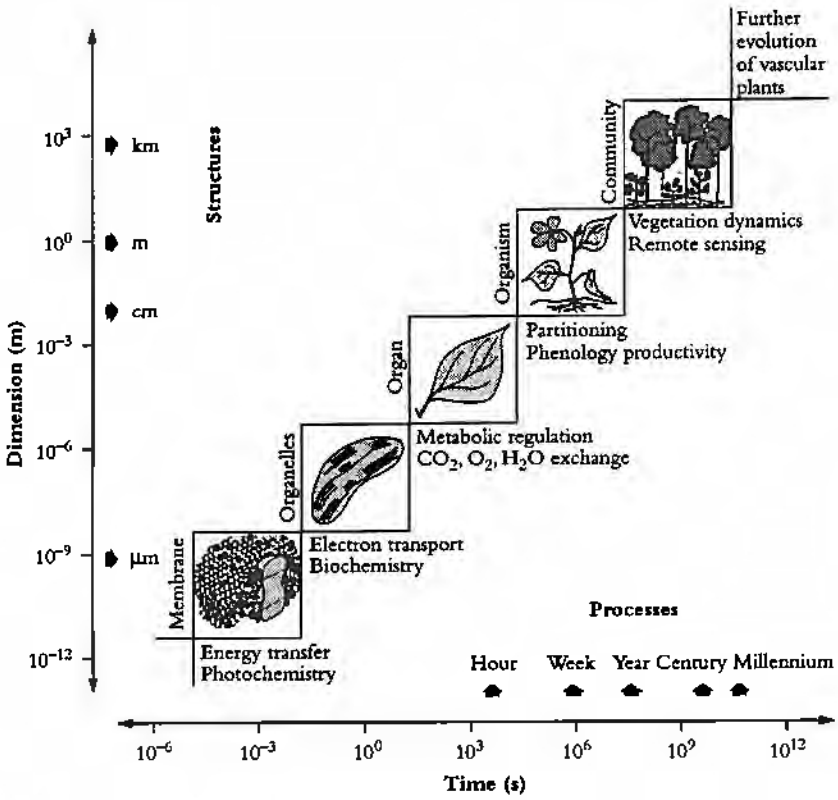


Figure I.2 Structures and processes in plant biology span dimensions from micrometres to kilometers, and time intervals from microseconds to millennia. Genotype × environment interactions apply at all these levels and are implicit in our analysis of short-term function in particular genotypes, as well as in our understanding of long-term adaptive change and hence evolution of new genotypes
(Based on Osmond and Chow 1988)

Soil–plant–atmosphere

Plants are wet inside, and with rare exceptions will die if they do not remain so. But this is not to say that their outside surfaces are necessarily wet. On the contrary, they are usually dry to your touch. If a drop of water is placed on a leaf it will usually sit there, precariously, roughly in the form of a hemisphere, because the leaf surface is hydrophobic. It is essential that the surface be hydrophobic and poorly permeable to water, for a free water surface can lose water at rates approaching 1 mm per hour on a hot day. An unprotected leaf would lose its entire water content within a few minutes at these rates of evaporation unless it had a prodigious ability to replace the lost water.

Plants originated in the sea and there faced little risk of drying out. They could not grow on land until they had evolved facilities both to control and to make good the evaporative losses from their leaves. Control is achieved partly by means of a cuticle, a poorly permeable layer that covers photosynthetic surfaces of plants, and partly by means of the stomata, variable pores in these surfaces, which largely prevent evaporation when shut and allow CO_2 assimilation to proceed rapidly when open. Evaporative losses from leaves are made good by roots, which can extract water from the soil, sometimes, if necessary, from very great depths, and by the vascular system, which contains conduits that carry the water from roots to transpiring leaves.

This book, *Plants in Action*, has much to say about leaves and their stomata, roots and vascular system, but it is worthwhile trying to imagine what other paths evolution might have taken in enabling plants to grow on land. Development of roots and a vascular system seems unavoidable, at least in higher plants, whose internal milieu must be much more strongly buffered against environmental changes than that of, say, lichens, or resurrection plants, those oddities that can dehydrate completely without losing the integrity of their cells and which can respond to rewatering so rapidly that they can be photosynthesising again within hours. But what of stomata? Why have them at all? Do plants really need to transpire? This question has several aspects to it, some of which have vexed plant physiologists for a long time. One aspect is this: plants grow by assimilating CO_2 from the air and fixing it in organic form

with the help of sunlight, that is, they photosynthesise. Evolution has not (yet?) provided them with a membrane that is permeable to CO_2 but impermeable to water vapour; therefore the outer covering of the leaves, which must be largely impermeable to water vapour, must have valves to let CO_2 in. Transpiration is an unavoidable accompaniment of the uptake of CO_2. Unavoidable if plants are to photosynthesise rapidly and thus keep up with their neighbours in the evolutionary stakes. However, there is one species, *Stylites andicola*, that has taken an alternative route. This plant has no stomata. CO_2 is taken up by its roots and is transported to leaves through continuous air passages in its roots and stems. This species grows in the Peruvian desert on an average annual rainfall of 30 mm with an interval between rains that often lasts for years. As might be expected, it grows very slowly, even when conditions are good (Keeley, Osmond and Raven 1984).

The point of this preamble is that active cells must be well hydrated and that evolution has produced various structures that enable higher plants to keep their cells wet enough to function — to divide, to grow, to photosynthesise, to transport, to respire, to excrete, to defend, to communicate — even though most of these cells are in the shoots of the plants and so are not only remote from their supply of water, but are also exposed to a very drying environment.

Concepts in plant science: an example from physics

This book uses many different conceptual frameworks for discussing how plants work. The reason for using many frameworks derives from the way that our minds apprehend the complex world around us by dividing it into a hierarchy of conceptual layers, each one nested within the one above like Matruschka dolls (consider, for example: subatomic particles, atoms, molecules, grains, bricks, walls, buildings, cities). Each of these conceptual layers has its own terms, ideas and principles, and much of what we call 'understanding' involves translating the terms and ideas of one layer into those of the adjacent layers. We can pour some concrete into this rather abstract mould by taking as an example the relationship between the ideal gas laws and the kinetic theory of gases.

The pressure, P, volume, V, absolute temperature, T, and number of moles, n, of an enclosed ideal gas are related thus: $PV = nRT$, where R is the universal gas constant. This relationship is useful in that it helps explain many everyday phenomena such as the behaviour of a bicycle pump and the leavening of baked bread. Moreover, this law can itself be explained, and the simplest vehicle for doing so is the kinetic theory of gases: we imagine that the gas consists of myriad molecules ($n \times$ Avogadro's Number), each of given mass but of negligible volume, moving randomly in an enclosed space, each with its own velocity which is changed only by perfectly elastic collisions with other molecules or with the enclosing wall.

The interesting thing about these two descriptions of a gas, one phenomenological and the other particulate, is that they appear, at first sight, to have little connection with each other. The ideas of volume and number are common to both descriptions, but not those of pressure, temperature and velocity. A molecule does not have a pressure or a temperature, and $PV = nRT$ does not embrace velocity. The two descriptions are examples of different conceptual layers, and each has terms and ideas that are peculiar to it. Yet the two layers are connected and the apparently disparate terms are related. The connection comes by considering the average properties of a large number of molecules because P is proportional to T and both are in turn proportional to the mean square velocity of the molecules.

This example of a connection between conceptual layers comes from classical physics, but note that the phenomenological discovery (gas laws) was made long before the particulate explanation (kinetic theory) was available. Indeed, this sequence is universal in any scientific exploration of natural hierarchically organised systems and the subsystems that comprise them. In effect, we need to know collective behaviour (e.g. $PV = nRT$) before we can ask pertinent questions about the parts (e.g. how can we best summarise the behaviour of a legion of elastically colliding molecules). The reason that we first need to appreciate integral behaviour of the whole system is that the problem of summarising the behaviour of the parts is underspecified. We need to specify at least two constraints when considering the general kinetic behaviour of gas molecules before we can derive the gas laws, namely, (1) there is a fixed number of molecules, and (2) they are enclosed within a bounding wall. The kinetic theory applies as well to the earth's atmosphere as it does to an enclosed gas, but then we need to consider different constraints. We replace the constraint of an enclosed space with that of an infinite space bounded internally by the earth's surface, and subject to a gravitational field. History has shown that in practice we do not become aware of the extra information we need, that is, the constraints on the behaviour of a subsystem (e.g. a molecule

or a cell) until we have considered the behaviour of the system as a whole (e.g. a gas or a tissue). This is a crucial principle that highlights the importance of exploring plant behaviour at all levels of organisation. Only by articulating connections between all the layers can we hope to have a comprehensive understanding of how plants work.

Functional analysis of plants

What then are the conceptual layers into which we divide plant science? To some extent these are arbitrary, but are commonly as follows: community, whole plant, organ, tissue, cell, organelle, membrane, molecule (polymer, monomer, gene) (some of which are shown in Figure I.2). These layers are essentially structural, but implied in them are processes occurring at a range of time scales, from geological, for evolutionary processes such as those that led to the facilities that are essential for plants to grow on land, through hours for cell division, to microseconds for conversion of radiant energy to chemical energy within chloroplasts.

In discussing how plants work it is useful to invoke the concept of function. This concept most clearly distinguishes biology from physics and chemistry, although 'function' is held to be indecorous by many biologists, for they see it as imbuing evolution with a sense of purpose that they deny exists. Whether or not evolution has a purpose is a philosophical question that is beyond the concern of this book, but there is no great difficulty with the concept of function in normal biological usage. It is simply a way of recognising that unlike simple physico-chemical systems that can be adequately studied using a linear train of thought, biological systems, when viewed at a long enough time scale, are recursive, as illustrated by the loop in Figure I.3. If the loop was not complete, the structures that comprise it would not exist. Clockwise flow around the loop represents analysis at progressively finer levels in the manner of physics and chemistry, but the loop is open for clockwise flow, and stops at 'Gene (frequency)'. Anticlockwise flow represents a process-based explanation of integral functions, and in terms of biological events is essential for closing this loop by producing the next generation. Functional explanations are ideas about the influence of given structures or processes on the ability of a plant to transmit its genes into the next generation.

Debates often occur between proponents of process and integrative explanations, and are generally specious. To understand biological phenomena thoroughly, and apply basic principles to commonplace situations, we need to analyse in the reductionist sense (clockwise in Figure I.3), as well as extrapolate from component processes in an integrative sense (anticlockwise in Figure I.3).

Accordingly, *Plants in Action* has adopted genotype × environment interactions as a fundamental theme for processes and adaptation in higher plants which leads to integrative explanations: processes in the sense that physiologists attempt to define the genetically determined workings of plants that have fitted them to a given niche in nature; adaptation in the sense that ever-changing environmental conditions impose an unrelenting selection pressure for genotypes with traits better suited to new conditions. A thorough knowledge of such processes can then underpin explanations of adaptation in nature and performance in cultivation.

Further reading

Passioura, J.B. (1979). 'Accountability, philosophy and plant physiology',. *Search*, **10**, 347–350.

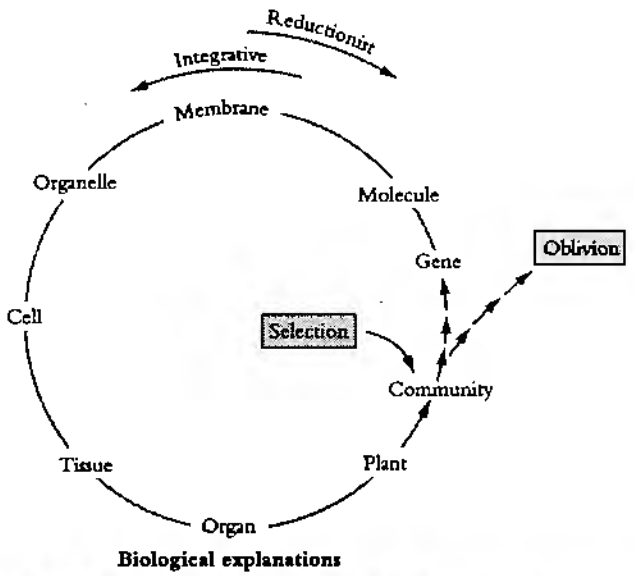
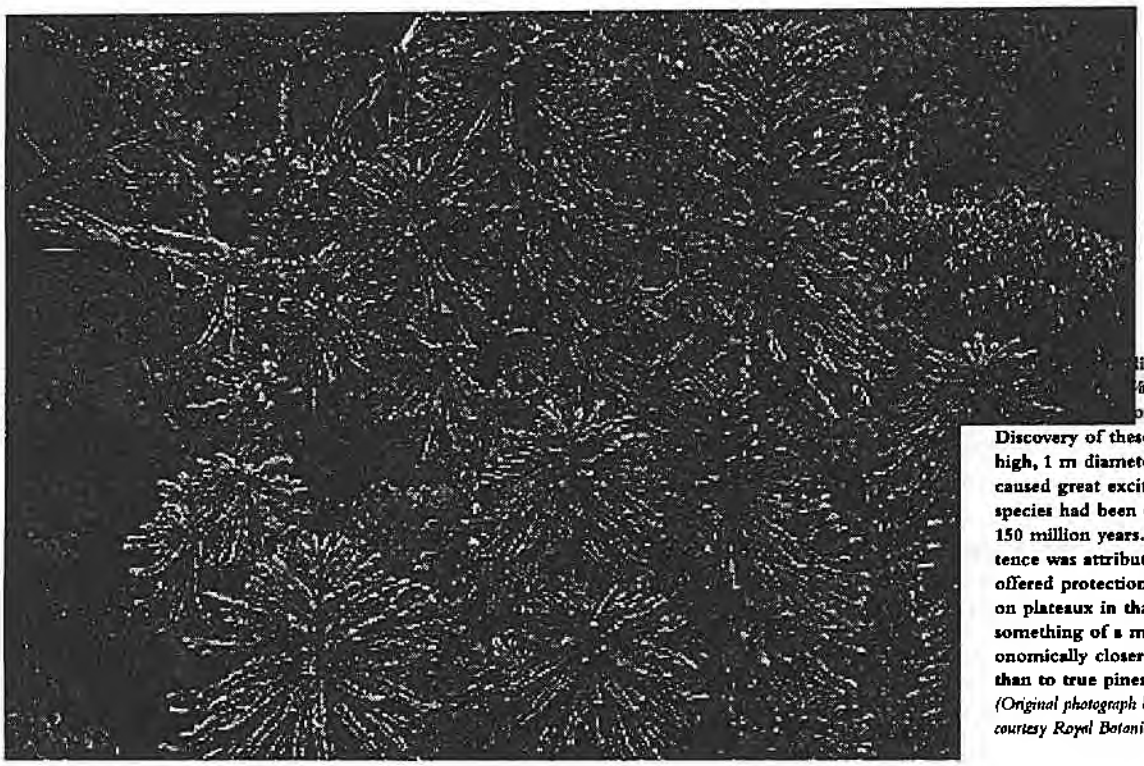


Figure I.3 A reductionist approach in plant science generates new knowledge at progressively finer levels of organisation. This sequence is shown here by progress in a clockwise direction from community via component parts to molecule and eventually gene. In principle, a knowledge of genotype × environment interactions at these various levels of organisation enables integration of process-based concepts. That sequence is shown here as progress in an anticlockwise direction from gene to community, and thence to the next generation; for without this loop being closed none of the structures within it would exist
(Original diagram courtesy J.B. Passioura)

A southern hemisphere view of nature

Don Adamson
SCHOOL OF BIOLOGICAL SCIENCES, MACQUARIE UNIVERSITY, SYDNEY



lic population of *Wollemia nobilis*) in a on northwest of Sydney. Discovery of these large trees (35 m high, 1 m diameter) in August 1994 caused great excitement because this species had been considered extinct for 150 million years. Their continued existence was attributed to a habitat which offered protection from fires common on plateaux in that region. 'Pine' is something of a misnomer; they are taxonomically closer to species of *Aumcaria* than to true pines (genus *Pinus*)
(Original photograph by Jaime Plaza, and supplied courtesy Royal Botanic Gardens Sydney)

Geology

The supercontinents of Gondwanaland and Laurasia began to disaggregate about 160 million years ago. Prior to this time, the southern hemisphere land masses and India were connected into a supercontinent, Gondwanaland. North America, Europe and much of Asia formed Laurasia. Gondwanaland was a southern hemisphere land mass. South Africa, Madagascar, India, South America, New Caledonia, New Zealand, Australia and various other fragments broke away and drifted northwards, leaving Antarctica behind. Australia and South America were the last major land masses to separate from Antarctica, Australia beginning slowly about 90 to 100 million years ago and establishing a deep ocean passage some 30 to 40 million years ago. Opening of the Drake Passage between South America and Antarctica completed an ocean connection around Antarctica, allowing development of circumglobal cold currents in the Southern Ocean and the thermal isolation of Antarctica from warm tropical water and air.

Plant life

Before this cooling there was a continuity of vegetation which only became fragmented as the fragments of Gondwanaland broke away from Antarctica. Angiosperms were already established worldwide as a major successful group of plants. The climate was warm, moist and conducive to plant growth even at high latitudes where availability of light in winter would have been no more of a problem than in the Arctic today. Forests were widespread, diverse and dominated by gymnosperms and angiosperms (presumably deciduous). As a result of the break up of Gondwanaland entire plant communities, containing representatives of the major types of plants now alive, were carried northwards. This inheritance can be seen today in many of the plants of southern hemisphere lands and is well illustrated by the distribution of major plant families such as the Proteaceae (banksias, grevilleas, waratahs) and Myrtaceae (eucalypts, bottlebrushes, ti-trees, lillipillis). Despite subsequent colonisation by long-distance dispersal of propagules and/or

merger with northern lands (South America with North America, Africa with Europe, India and Australia with Asia), the floras of the continents and islands of the southern hemisphere remain more closely related to each other than they are to northern hemisphere floras derived from fragments of Laurasia.

As Australia accelerated northwards from Antarctica about 40 million years ago it moved into warmer climatic zones. Cool temperate forests, abundant over most of Australia, became confined to wetter areas. The vegetation as a whole became more open and in places heathlike.

Until about 15 million years ago one of the most abundant and widely distributed angiosperms in Australia was *Nothofagus* (southern beech). Evidence from a site in the Lachlan region of central New South Wales indicates that the decline in abundance of Nothofagus was accompanied by a corresponding increase in pollen from eucalypts and other mainly rainforest species from the same family (Myrtaceae). Somewhere between 15 and 8 million years ago the amount of charcoal relative to pollen at the same site also increased. Most likely, communities of eucalypts and related genera were subject to more frequent burning than the *Nathofagus*-dominated forests that preceded them. Similar evidence from pollen profiles from Lake George near Canberra reveals an abrupt change from a mixed forest of *Nothofagus* and gymnosperms to an open shrubland with grasses and daisies about three million years ago.

From the Gondwanan stock, and by evolution of new life forms, plants adapted to match emerging conditions of low and unpredictable water supply, strong sunlight, higher temperatures and frequent burning. The new combinations of environmental conditions produced the vegetation types which now dominate the Australian continent. Genes for many of the adaptive attributes were presumably already present, scattered through the Gondwanan flora as a consequence of prior exposure of ancestral stock to environmental stress associated with life on land.

During its drift northwards Australia was subject to only relatively minor geological disturbance although some volcanic activity occurred on the eastern side of Australia and large parts were inundated by shallow seas which for long times isolated southwestern Australia from eastern Australia. Such isolation aided evolution of a remarkably rich endemic flora in southwestern Western Australia. The sea retreated, and limestone soils may have sustained the isolation; developing aridity certainly did so.

By contrast, New Zealand after its separation from Antarctica and Australia some 60 million years ago has had a tumultuous history of geological disruption involving partial or possibly complete inundation by the sea, severe volcanic activity and glaciation. New Zealand vegetation, like that of Australia and other southern continents, also changed in response to climatic change and environmental stress. As New Zealand broke away and moved north conifers became less abundant and a mixed evergreen angiosperm and gymnosperm flora developed. A change from *Nothofagus*-dominated rainforest to drier vegetation occurred in the Miocene around about the same time as there was clear evidence of drying in Australia. Today, conifers are again abundant in rainforest communities and the climate is wetter due to New Zealand's present location across the path of moist oceanic winds.

Biogeography

Because of global position and stable geological history, Australia is dry and intensly weathered. In addition, large areas are covered by sedimentary rocks whose minerals have already been through at least one cycle of weathering. There has also been negligible glacial activity to grind rock and expose unweathered minerals. As a result, most of Australia offers plants with a poor supply of both water and nutrients. Moreover, plant communities are also subject to burning by fires ignited by electrical storms.

In exploring the question of adaptation of plants to aridity, fire and mineral deficiency, note that only two genera (*Eucalyptus* and *Acacia*) dominate the top stratum of vegetation over three quarters of Australia. Eucalypts occur in open forests and woodland. Acacias occur in nearly all plant formations but are particularly prominent in semi-arid and arid regions. Both genera are obviously very well adapted to present-day conditions; both are of ancient Australian origin; both are sclerophyllous (i.e. have leathery/rigid leaves); both could be described as scleromorphic (extremely woody). The same could be said of many other typically Australian plants.

Water shortage is such an obvious limitation to plant growth in Australia that it is easy to conclude that specialised plant structures and behaviours are adaptations to conserving water or surviving under water stress. Scleromorphy in the Australian flora can be interpreted as an adaptive response to an increase in aridity and/or low nutrient supply.

Scleromorphy is also important for fire ecology. Scleromorphic vegetation provides a mass of dry woody fuel that is very slow to decay. Decay of lignin by microorganisms

occurs much more slowly than decay of most other plant products. Consequently lignin-rich plant litter (dead leaves, branches, fruit and bark) accumulates faster than it decays. Fire therefore becomes inevitable and increasingly severe as litter accumulates. Many plants, such as eucalypts, paperbarks, ti-trees and boronias, also accumulate large amounts of volatile flammable oils in their leaves. During hot fires the canopy produces an explosive mixture of vapourised oil. Sclerornorphic vegetation promotes its own burning. Consequently only plant species that have developed characteristics that allow their individuals or their seeds to survive fire will survive. Not only does sclerornorphic vegetation promote fire, but some species are so coupled to a fire-prone environment that they would probably become extinct without periodic burning.

Charcoal in ancient sediments implies that fire has been a feature of the Australian landscape for a very long time. However, the fire regime of the whole continent must have begun to change when humans first occupied Australia at least 50 to 60 thousand years ago. Fire was used for purposes of hunting, safety and access to the countryside. Fire is a frequent event across all woodlands, shrublands and grasslands of Australia and all plants beyond rainforests are adapted to cope. Replacement of the leafy canopy after fire by sprouting of dormant buds is one of the main adaptations which allow well-insulated plants to survive in a fire-prone environment.

The history of Australian vegetation since the break up of Gondwanaland provides some insight into the magnitude of the changes that have occurred in relative isolation from the rest of the world and the nature of the selection pressures that have operated. On a time scale of millions of years, cool temperate forests that covered much of the continent when it was 60°S were replaced by open forest, woodland, shrubland and grassland. Those communities were better suited to the warmer and drier conditions of its present location.

Human impact

In stark contrast to geological events, and on a time scale of only decades since European settlement, 70% of Australia has been turned over to agriculture and forestry. Almost all potentially productive and marginal farming and grazing land has been cleared. Soil degradation, including wind and water erosion, acidification and salinisation have become severe national problems. Large numbers of pest species, both plant and animal, have emerged from the huge numbers of newly introduced organisms while many indigenous plants and animals are extinct or endangered. Much of the Australian landscape is not sustainably productive, having been exploited for up to 200 years. Such 'mining' of vegetation and soil continues unabated despite wide recognition of precious 'captial' that is irretrievably lost in the process.

New Zealand faces its own set of severe problems including plant and animal extinctions, rapid spread and dominance of pest species ranging from European gorse to Australian brush-tailed possums which, by selective browsing, are threatening extinction of some native plant species. New Zealand has been severely over-cleared of native vegetation even on marginally productive land. Steep slopes have been destabilised and some types of vegetation have been lost.

Southern hemisphere plants are worth preserving as a heritage from the distant past, as vegetation for the future, and for numerous practical present-day purposes ranging from erosion control to sources of useful chemicals. In Australia and New Zealand substantial areas have been set aside for the protection of vegetation but in both countries some types have disappeared entirely and others are unrepresented in the reserves. However, compared to many countries which have much less of their original vegetation to conserve, Australia and New Zealand are well off.

The basic tenet of *Plants in Action* is that every facet of a plant's existence is an outcome of genotype × environmental interactions. In present times as distinct from geologic past, humans must now be included as a significant environmental factor for that interaction.

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Crop adaptation in Australasia

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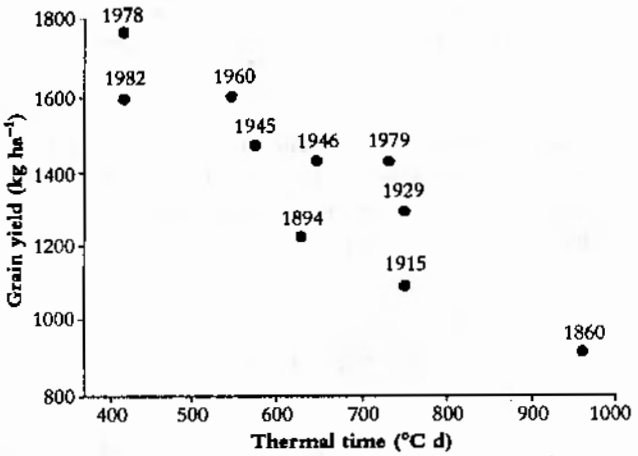

Figure I.5 A practical consequence of early flowering. Selection of Western Australian wheat varieties for higher yield over the past century has been highly successful, and associated with a reduction in thermal time (in degree-day, °C d) to the first stage of the flowering process *(Redmon from Richards 1991)*

Origins

When European settlement came to Australia late in the eighthteenth century, it was the only continent without any agriculture and lacking indigenous crop plants. The Aboriginal hunters and gatherers knew well which plants could be gathered for food, fibre or medicine, and when and where. They also practised some agronomic management such as broadcasting millet seed, replanting yam tubers or burning off yam or macrozamia tops. However, when the first fleet arrived in early 1788, they brought a variety of plants, fruits and seeds either from Britain or acquired in Rio or Capetown along the way. A few acres of barley were sown near the site of the Botanical Gardens soon after arrival but they gave a pitiful crop barely returning the amount of seed sown. The next attempts to grow wheat, barley and other cereals were shifted to better soil at Parramatta. Even there, returns were poor. Governor Phillip saw the problem as lack of agricultural expertise among the convicts: 'If fifty farmers were sent out ... they would do more in one year in rendering this colony independent of the mother country ... than a thousand convicts'. However, the low yields persisted even after the farmers arrived in 1793, and a botanist in 1800. The real need was adaptation by introduced crops to Australian environments.

In New Zealand the initial problems were less severe, partly because the Maori had already introduced and cultivated several crops and because the soils were more fertile and the climate more suited, at least in some areas, to European crops and pastures. The Canterbury Plains were more readily converted to agriculture, and British grasses such as cocksfoot (*Dactylis glomerata*), timothy (*Phleum pratense*), perennial ryegrass (*Lolium perenne*) as well as white and red clovers (*Trifolium repens* and *T. pratense*) flourished widely. Their adaptation has of course improved since then by regional selection, but some English cereal varieties were still in use in the 1930s.

Plant introduction

Not all domesticated plants from Europe required adaptation to Australian conditions. Blackberry (*Rubus fruticosus*) spread quickly in Victoria (with some help from Baron von Mueller) while *Lantana camara* (possibly introduced by Phillip) soon became a nuisance in warmer areas. Many serious weeds were introduced unwittingly such as Bathurst and Noogoora burrs (*Xanthium spinosum* and *X. pungens*) as were several useful grasses, clovers and Townsville stylo (*Stylosanthes humilis*). Given the ready adaptation of these and many other introduced plants to Australian conditions, why were there problems with many of the temperate cereals and seed legumes?

Crop adaptation

By and large, temperate cereals and seed legumes grew well enough. One farmer wrote of his crop: 'On the 21st of October (1803) a more beautiful appearance of a successful harvest never flattered the expectations of a farmer ...' But in the end the crop was not worth reaping. In this case the cause was rust, but as often it was water stress. Even when autumn sown, the predominantly British varieties tended to flower too late, with the result that seed development occurred in the hottest and driest months. Governor Phillip's first sowings of wheat in May 1789 were harvested in December, and his barley sown in August was harvested in January. Had Australia been colonised by a Mediterranean country, with Mediterranean cereals and legumes, the problems of crop adaptation would have been less acute.

Nevertheless, early Australian agriculturists persisted with English varieties of wheat that normally ripened in late July, and coinciding with harvest festival in Britain; varieties which, as Farrer said, 'yielded more of disappointment than of profit. Too late in ripening for our climate, their ears are blasted and their grain pinched by the hot winds of our summers.'

From the 1860s to the 1890s the most widely grown wheat variety was Purple Straw. Previously thought to be a selection from an English variety, Wrigley and Rathjen have now shown it to have been selected from a Tuscan wheat sent from Italy to Scotland and thence to a South Australian farmer. Similarly, the varieties which replaced Purple Straw in the 1890s derived from farmers' selections in South Australia from non-English varieties. Ward selected rust-free plants from a South African variety to give 'Ward's Prolific', from which another grower selected the earlier-flowering 'Gluyas Early'. At about the same time Steinweidel selected tall, early-maturing off-types of an American variety to establish the wheat bearing his name.

Cereal breeding

Although A.B. Robin of South Australia began wheat hybridisation in Australia, it was the purposeful and comprehensive crossing program begun in 1889 by William Farrer of Lambrigg, now almost enclosed by the expansion of Canberra, that transformed crop adaptation in Australia.

The two crucial elements in Farrer's success were his explicit formulation of his goals as a plant breeder, and the international scope of the wheat varieties used in his breeding program. The clarity and comprehensiveness of his objectives were sharpened in a scathing series of exchanges he had in 1882 with two newspapers, *The Queenslander* and *The Australasian*. By the time he discussed them at the Australasian Association for the Advancement of Science in 1898, they had become a well-honed list which included not only resistance to the various rust diseases and high baking quality but also such physiological features as earlier ripening, shorter, stronger straw, upright narrow leaves, reduced tillering and better grain retention. Farrer was also the first to draw on the wheat gene pools of the world to attain his aims. As the great Russian plant geographer, Nicolai Vavilov, acknowledged in referring to Farrer's breeding program in 1935: 'There is probably no region where intraspecific and interspecific hybridization of wheat has been so extensive as in Australia'.

Well before Mendel's paper was rediscovered, Farrer had stated that he selected the combinations of characteristics he sought from 'the variable generation' (i.e. the first selfed generation from his initial crosses), particularly for 'that delicate and obscure but most important quality ... which we include in the inconvenient term of constitutional fitness for the locality'. Early ripening was a crucial component of such fitness, which Farrer derived from Indian varieties, and which helped his wheats to escape rust. Farrer suggested in 1898 that earlier ripening was also drought-escaping and 'might be the means of extending some of our cultural industries, and certainly that of wheat-growing, appreciably further inland', and that much could 'be done by means of artificial selection to expedite the work of acclimatization'.

Indeed, wheat breeding since Farrer's time has continued many of the trends he initiated. Progressively earlier flowering in more recent wheat varieties continues to be apparent in several states as illustrated for Western Australia in Figure I.5

(see Figure 5 in Richards 1991). In trials at any one site there is often a sharp optimum 'time to ear emergence' in relation to yield, which has become shorter as plant breeding and crop management has improved. This optimum time is strongly influenced by seasonal incidence of drought and heat in each locality, but also by other factors such as frost injury. To date, much genetic manipulation has been empirical, involving the inherent earliness of varieties and their responses to vernalisation by low temperatures and to seasonal changes in daylength. These are 'the delicate and obscure' adaptive processes to which Farrer referred, and there is still much to be learned about their compensating interactions in the determination of yield (Section 6.4). Moreover, as economic and agronomic conditions change, earlier trends in adaptation may even be reversed. For example, in breeding of feed wheats for higher rainfall zones, emphasis is being placed not only on red grains but also on later-flowering winter varieties with an English background of the kind rejected by Farrer. Crop adaptation is a continually shifting process.

Temperate pastures

This account of the early adaptation of wheat to the more Mediterranean and lower rainfall environments of southern Australia is illustrative of the adaptation of other crops and pasture plants in these regions. With a pasture grass such as *Phalaris tuberosa*, which probably entered Australia via the Toowoomba Botanical Gardens in 1884, and whose value was recognised after its escape from there, it was more than 60 years before expeditions to the Mediterranean region deliberately collected seeds over a range of altitudes and environments for the purpose of enlarging the gene pool for a breeding program for improved adaptation.

With subterranean clover (*Trifolium subterranean*), on the other hand, there were probably many unwitting introductions from the Mediterranean region in grass seed samples. This is implied by the great range of growth characteristics and flowering times found among the many locally adapted varieties. Such variation was noted in subterranean clover after its value as a pasture plant and soil-fertility restorer was recognised by A.W. Howard of Mount Barker. In warmer areas with short growing seasons, only very rapidly flowering varieties could set seed and survive. At sites with longer growing seasons, the earlier-flowering varieties were outcompeted by later-flowering ones which grew larger and set more seed. In general, therefore, later flowering in a local variety was an adaptation to a longer growing season. As with wheat, early flowering in varieties of subterranean clover such as Dwalganup is associated with inherent earliness (evident in an absence of chilling requirement and relative indifference to daylength). Late-flowering varieties such as Tallarook have a requirement for vernalisation and/or long days. In this instance, natural selection had ensured a close adaptation of genotype to habitat which gave rise to the impressive range of local varieties in southern Australia.

Rice

Before we turn our attention northwards, two other examples of southern adaptation should be considered. Attempts to grow rice go back to at least 1869 in Queensland but yields remained low even after irrigation was introduced, particularly because of severe weed problems. In the irrigated areas of New South Wales beginning around 1922 it was a different story. Short-grain varieties from California such as Caloro, and later their longer grain varieties such as Calrose, followed by varieties bred from them in Australia, proved well adapted and plantings expanded rapidly. In fact, the varieties and conditions — especially the clear, sunny days and cool nights — were so well matched that for many years Australia had both the highest national average rice yields and the world record crop yield for rice.

Lupins

The other example of southern adaptation comes from Western Australia where a naturalised species of lupin introduced as a forage plant from overseas (*Lupinus cosentinii* or *digitatus*) was domesticated by John Gladstones who selected lines with reduced pod shattering and earlier flowering. In this case the crop was well adapted to the local environment. Further selection will be required to extend the range of lupin crops to other regions of Australia and overseas.

Sandplain lupin is not endemic to Australia, whereas wild relatives of rice, sorghum, cotton, soybean, tobacco, yams and other warmer climate crops are. Wild relatives pose problems for pest and disease control in crop species, but also offer a possibility of closer adaptation to Australian conditions if not to others. For example, Kanaka cane cutters partly domesticated a wild Australian yam, *Dioscorea transversa*, while in Queensland, in an effort to replace their own noble yams. When taken with them back to Melanesia on their return, the Australian species failed to form tubers and was rejected because it 'does not know the seasons'.

Sugar cane

Knowing the seasons, through their responses to daylength, is an important component of crop adaptation at low latitudes. Although sugar cane was brought to Australia by the First Fleet, it did not become established until reintroduced from Tahiti about 30 years later. However, adaptation to North Queensland

conditions began in earnest at the end of the nineteenth century when collecting expeditions to New Guinea brought back several *Saccharum officinarum* clones which were used by the Queensland Acclimatisation Society, the Colonial Sugar Refining Company and, later, the Bureau of Sugar Experiment Stations in selection and breeding programs. Many locally adapted varieties were produced, more than 50 of which were being used in 1971, although a small number of widely adaptable varieties accounted for half of the sugar production.

Cotton

There are nine wild species of cotton (genus *Gossypium*) endemic to Australia, but this crop was not grown on a significant scale until the American Civil War, and became a major crop only when extensive irrigation areas became available. In the Murrumbidgee Irrigation Area the growing season proved to be too short and too cool for profitable production, and most Australian cotton is now grown in the Namoi Valley. Although American varieties have yielded well in that area, better-adapted Australian-bred varieties are now being grown to a greater extent.

Soybean

Soybean is another warmer zone summer-growing crop with many wild relatives in Australia, but was of only minor significance in Australia until the 1970s. As with other irrigated crops, soybean varieties bred elsewhere can be highly productive when their responses to daylength and growing season are appropriate. Availability of varieties adapted to the southern areas of the USA was a key factor in the expansion of the soybean crop in Australia. Even-later-maturing varieties are being bred for north Queensland conditions where local adaptation calls for greater sensitivity to daylength.

Tropical pastures

Just as different crops have been adapted to northern Australia, different pasture plants were also required which were beyond the adaptive range of British and Mediterranean grasses, clovers and medics. Indeed, the search for grasses and forage legumes suitable for cattle grazing in northern Australia was an unprecedented adventure in acclimatisation.

An unwitting introduction of Townsville stylo (*Stylosanthes humilis*) in 1904 showed the way. Stylo productivity in semi-arid conditions induced N. Pollock (Queensland Department of Agriculture) to throw seed out of train windows wherever he travelled in the state. However, stylo competed poorly in many parts of Queensland, as did several other forage legumes adapted to wetter conditions, and in 1947 an expedition to four South American countries was organised by the CSIRO to collect grasses and legumes likely to adapt to northern Australian conditions. So well adapted did several of the legumes brought back prove to be that the carrying capacity of Queensland pastures was transformed by their introduction.

Understanding crop adaptation

Although Governor Phillip, in 1789, had not recognised the need for the adaptation of crop and pasture plants to local conditions, various state acclimatisation societies (beginning in Victoria in 1861 and in New Zealand in 1864) sought to spread knowledge of acclimatisation and to understand the causes of the success or failure of introduced plants. So soon after the publication of Darwin's *Origin of Species* in 1859, they were often hampered by a belief in the fixity of species and in their perfect adaptation, as well as by ignorance of the crucial role of such processes as vernalisation and photoperiodism. Yet just as Farrer was an effective plant breeder before Mendel was rediscovered, so also could he select for earlier maturity without knowledge of the effects of daylength on flowering. Nevertheless, such knowledge has greatly increased our power to screen plant introductions or breeding materials for particular environments, together with our continually improving knowledge of local soils and climates. Yet there is still a great deal to be learned about interactions between genes and environments, about physiological states such as inherent earliness of flowering, about the factors determining local adaptation and, as importantly, about why some genotypes have much wider adaptability than others. Plant adaptation is still a relatively dark continent on physiological maps.

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Plant science applied: a case study on cotton

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Figure I.6 Cotton harvest in progress at Narrabri, New South Wales (July 1995)
(Original photograph courtesy P.E. Kriedemann)

Origins and domestication

Worldwide, the genus *Gossypium* consists of over 40 species of perennial xerophytic shrubs (including domesticated cotton, *G. hirsutum*). They are frost-sensitive short-day plants found along banks and beds of dry streams. Though the genus is pantropical, individual species have limited distribution and are of relic status with little genetic diversity, suggesting a declining genus. All species except two are diploid. The diploid species are divided into five genomes, each of which is largely confined to one continent. Australia has a rich flora of wild species, some very rare, but none has contributed to commercial varieties so far.

Cotton lint consists of seed hairs that collapse in cross-section and form convoluted ribbons when mature allowing them to be spun into yarn. Apart from *G. herbaceum* the other wild species have the stiff seed hairs that cannot be spun. Traits appropriate to spinning were selected after domestication and allowed cotton to be grown in temperate regions, together with selection for longer and finer fibre and heavier yield. Domestication did not involve loss of sensitivity to daylength as a trigger for reproductive development. Moreover, adoption of an inherent annual habit was not required because termination of a cropping cycle can be imposed by withholding irrigation in combination with defoliation.

Water Relations

Cotton is grown in the Yemen in a way that offers invaluable insight into the ecology of cotton's wild ancestors, and helps explain the behaviour of modern crops. Erratic floods in the Yemen are impounded in order to store the water in their deep alluvial soil. Cotton is then grown entirely on this stored water. Such conditions are remarkably similar to the natural environment of wild ancestors of cotton, and strikingly illustrated by the occurrence of an indigenous wild species as a weed in cultivated crops, both crop and weed utilising similar adaptive features. The root system explores the soil to the depth wetted, and an indeterminate shoot develops at a rate unaffected by the amount of stored water, until approximate-

ly three-quarters is exhausted, whereupon morphological development and vegetative growth stop abruptly, and the crop matures fruit which have already set. Plant size and fruit number thus depend on the duration of development, which in term depends on how long it takes for the water to run out.

This pattern of development is well adapted for survival in arid and semi-arid environments where the water supply from rainfall or floods is erratic and can vary greatly from one season to the next. An indeterminate habit allows plants to make full use of variable water supplies by growing large or small according to water supply. Cotton plants appear to recognise a signal indicating that soil water supply is running out. They then stop development, shed young fruit and mature fruit already set. Passioura *et al.* (1993) have accumulated evidence from several sources that plants react to drying soil in response to signals from their roots. Contrasts between rainfed and irrigated cotton are variations of this basic pattern. In irrigated crops, a succession of drying cycles replaces a single prolonged cycle of rainfed crops.

There appears to be another signal at the wet end of the range that triggers the rank growth syndrome. Most bolls are shed, unable to assert their priority for assimilates, while vegetative growth is excessive indicating no shortage of resources. This is presumably a latent response from wild progenitors to maximise vegetative growth and delay setting fruit when conditions warrant. Both signals are keys to managing cotton crops.

Analysis of yield

Yield response to growing conditions is associated mainly with variation in the number of mature fruit produced. Two distinctive features of the plant appear to be involved: an indeterminate habit and a propensity to shed fruit under stress. As a consequence of an indeterminate habit, plants produce flower buds in a regular and orderly way. Rate of flower bud production is predictable, but duration is not. Production of mature bolls then tracks along behind bud production but rate of boll production is not predictable due to variable shedding.

As a student I had been taught that it was not enough to simply apply treatments and measure yield. Yield components should be measured and 'developmental studies' done during the season. This mainly consisted of counting things like branches and fruit and flowers buds. I accumulated a lot of data and they did not help explain yield.

Then I discovered crop physiology! I read Watson (1952) whose aim was 'to analyse yield in terms of antecedent growth' because 'this was the kind of knowledge that can be put to practical use in crop husbandry'. Here is science rolling up its sleeves! I was also strongly influenced by Hugh Bunting (1958) who introduced crop physiology as a subject at my Alma Mater in the decade after I was a student. Watson dismissed 'developmental studies' and argued that it was 'more logical to base an analysis of yield on weight changes that occur during growth than changes in morphological character'. I readily agreed — and did crop physiology on cotton, analysing increase in dry mass in terms of the size and efficiency of the energy capture system, in other words, the crop canopy. I was confident I would soon understand yield in cotton. When Professor Donald challenged us to use physiological criteria in crop breeding instead of pure empiricism, I wrote with misplaced confidence to assure him that for cotton it would soon be done! I was wrong. I accumulated a lot more data and not much more understanding.

Although Balls' cotton research was seminal in the history of crop physiology (he coined the name), and although others followed in his footsteps (reviewed by Watson 1952, Evans 1975 and Wilson 1992), we seemed to have lost our way. Looking back, I can see two distinct lines of investigation: (1) study of development and (2) analysis of dry mass increase. I call the study of development 'a numbers game', as opposed to 'weight watching'. Their respective proponents rarely made contact!

There are two reasons for a preference for weight watching in crop physiology. First, weight watching tends to emphasise similarities between species and allow generalisation, whereas study of development tends to accentuate differences. Second, weight watching carries an implicit assumption that because the major part of yield consists of products from photosynthesis, then a study of photosynthesis would inevitably lead to an understanding of how yield is determined. This is not necessarily so.

After noting voluminous studies on growth of cotton over the previous 60 years, Stern (1965) commented 'In spite of the large amount of information available, there is little from which general principles may be deduced to predict the behaviour of the cotton crop in a new region', or indeed in any region. The more we knew, the less we understood.

On those rare occasions when weight watchers had played the numbers game, that is, when development and growth were studied together, research outcomes were fruitful. Mason (1922) postulated that a cotton plant retained as many fruit as it could supply with nutrients (mineral and assimilates). This

has become known as the Nutritional Theory of Shedding and has taken a fair battering over the years, particularly at the hands of hormone physiology. Nevertheless, this theory has survived virtually unscathed and is fundamental to understanding the dynamics of fruiting, and is incorporated in all cotton simulation models.

Cotton in Australia

Cotton had been grown in Australia experimentally from time to time in the first half of the nineteenth century, but it took reverberating events in the USA to induce large-scale production in Australia. A world shortage of cotton during the American Civil War and later depredations of the boll weevil, exacerbated by shortage of foreign exchange after World War I, stimulated production of rainfed cotton in Queensland. Additionally, in the early 1960s restrictive US Farms Bills persuaded Californian growers to bring their methods of intensive irrigated production to Australia. So began the modern Australian industry. Over the next 30 years the industry expanded dramatically and in the course of 15 years Australia swung from being an importer of cotton to being the world's fourth largest exporter. Cotton ranks third in Australia in value as an export crop.

I came to the Ord Valley in 1970 in time to witness the ecological disaster when *Heliothis armigera* became resistant to DDT (genus subsequently renamed *Helicoverpa*). I was, and still remain, challenged by the similarity of environments in the Sudan Gezira and the Ord Valley in terms of both climate and soil. I was impressed by Norm Thomson's varietal work which showed that adaptation to the economic environment (intensive mechanised production) was more important than adaptation to the physical environment (soils and climate). Nevertheless production was abandoned on the Ord, but continues in the Gezira. I always thought research was abandoned prematurely; it was like cowardice in the face of enemy fire. *Heliothis* said 'boo!', and we all ran away. It is a source of great satisfaction that cotton research has been resumed on the Ord with new technology and a lot of hard-won wisdom. Intensive cotton production in the tropics is a great challenge, being afflicted by an interaction between pests and rank growth and is rarely successful. Synthetic growth substances and transgenic varieties now offer hope of success.

Narrabri introduced me to temperate production. Cotton thrives in the irrigated valleys between the 22nd and 32nd parallels in eastern Australia. The crop has brought much prosperity and wealth to those valleys, to their towns and to individuals who live there, and even to the nation as a whole. Such are the economic realities. But when we consider the ecological realities, cotton is a disruptive crop. Production on the vertisol plains is intensive, highly mechanised, with heavy inputs of nitrogen (up to 200 kg ha^{-1}), irrigation water (up to 9 ML ha^{-1}), and pesticide (one or two herbicide and 8 to 10 insecticide sprays). Given such requirements, is cotton production sustainable?

Cotton management models

Cotton was threatening to become an ecological pariah in the 1970s. Heavy use of pesticides and irrigation water for cotton growing was of great concern. We needed a simulation model to explore options for pest and irrigation management at tactical and strategic levels. At a tactical level we wanted to use fruit counts to evaluate the potential for pest damage during the season. At a strategic level we wanted to identify the 'best bet' strategy for using limited irrigation water supplies in the face of uncertain rainfall. We needed to extrapolate the results of our experiments in a few years and locations to any year and any location in the cotton growing regions. OZCOT evolved as a simulation model for management of cotton crops.

I had tried first to build my own model. I realised I was trying to reinvent the wheel and used other people's models. I also rejected these as they needed too many inputs as well as local calibration. We eventually agreed with Conway (1977) that simulation models at that time were of little value in tactical pest and crop management (Hearn and Room 1979). We used a non-dynamic trajectory of yield development consisting of the number of fruit needed at any time in the season to achieve a specified yield by a specified date. Actual numbers were compared with the trajectory to determine how much pest damage could be tolerated.

The fruit model SIRATAC pest-management system was then built in a succession of steps (Hearn and da Roza 1985);

1. The number of counted fruit that would survive and contribute to harvest was estimated. The day-degree requirement for fruit development was used to build an age profile of the fruit counted. The proportion of young fruit shed was estimated as a function of the fruit load (the number of older fruit).
2. Production of flower buds was estimated as an empirical function of the cumulative number of flower buds and the fruit load. The former provided positive feedback and the latter negative feedback. The form of the function took account of the geometry of the branching structure.
3. The model crystallised round the concept of carrying capacity, which is the fruit load that reduces the rate of bud production and rate of fruit survival to zero. The concept implicitly incorporates the carbon economy of the crop; the ratio of fruit load to carrying capacity is a surrogate for the carbon demand:supply ratio for fruit.

The result is a simulation model that is simple and elegant, and which captures the dynamics of cotton fruiting when water and nitrogen are not limiting. It saw more than 10 years service in

the SIRATAC pest-management system.

We had asked 20 years earlier, 'Why does the crop close its doors, and why do some bolls drop out?' In this models we are saying, 'because the older bolls compete against other sinks for the limited assimilate supply, and are successful'. Twenty years previously we could not predict when the crop would stop producing flower buds and how many fruit would shed, or whether the crop would compensate for pest damage. Now we could. We had no more data than when these questions were asked. We had more relevant understanding. We did not need data on assimilate supply and demand. What we needed were concepts of competition among sinks for limited supply of assimilates, and the priority of the older fruit over other sinks including young fruit and buds generating more fruit.

The OZCOT and hydroLOGIC models were developed from the SIRATAC fruit model by linking it to the Ritchie (1972) water balance model for use in situations where water and nitrogen are limiting. Ritchie's model had already been used with a stress-day yield function to analyse strategies with limited water supplies (Hearn and Constable 1984). A leaf area generator, a boll growth model and a rudimentary soil nitrogen model were added, and photosynthesis included explicitly. OZCOT and hydroLOGIC have been widely used as management tools (Hearn 1994).

A new model, CERCOT, has been built by linking OZCOT with the soil water and nitrogen models from the CERES family of models, in order to simulate soil and plant nitrogen dynamics more realistically. Light interception and conversion to dry matter are done in the way pioneered by Monteith and followed in CERES, and partition is linked to the fruiting dynamics. CERCOT is currently in the final stages of validation.

Our modelling has thus gone a full circle. We started by turning our backs on simulation models and using a static model. A simple dynamic model was then built which has been made progressively more complex over the years. At no stage was it more complex than it needed to be to solve the problems being addressed.

Concluding remarks

Cotton is always fascinating and sometimes maligned, a crop that has shaped the history of many countries. Cotton is grown in a remarkable range of environments and economic circumstances from tropical to temperate, and from small-scale subsistence holdings to large and intensive corporate farms. Ther has been a dark side to cotton — slavery, colonial exploitation and alleged environmental vandalism. It is a crop that our media and environmentalists love to hate, a crop alleged to exhaust soil and require excessive amounts of insecticides and water; and yet this crop clothes humanity in natural fibre!

Ewqually for plant science, empiricism in cotton growing has given way to process-based models of growth and reproductive development as an aid to management on a huge scale. Such application of basic principles from crop physiology takes on added significance for a species that natural selection honed as a perennial, but which farming practice now manages as an annual. Moreover, gene × environment interactions are still at work on *Goossypium hirsutum*, but this time human selection for inherent earliness with wide adaptability to photoperiod will help shape future genotypes.

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Synopsis

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> When we plant physiologists are asked...to justify public support for our research, most of us would, I imagine, invoke its potential value to agriculture and horticulture...scratch a plant physiologist as many a sceptical review committee has done, and you find an agronomist, plant breeder or horticulturist.
>
> *(Evans 1977)*

'Adaptation in nature and performance in cultivation' now emerge as connected themes for *Plants in Action*. As demonstrated here in Part I, successful adaptation in nature does not necessarily imply satisfactory performance in cultivation. This arises because selection in nature results in traits that do not coincide with the performance requirements of agriculture or other forms of intense cropping. Overall rates of carbon acquisition and the scale of photoassimilate partitioning into reproductive organs (harvest index) are often sacrificed in nature in favour of devices for survival. This is especially evident over much of Australia where natural selection pressures commonly relate to environmental stresses, and native plants have evolved accordingly. Adaptation in our post-Gondwanan flora to drought, fire, nutrient stress and soil salinisation has attracted special attention by ecophysiologists.

Aboriginal Australians made extensive use of these species over several millennia of occupation, and had long since come to terms with the vagaries and seasonal limitations of our endemic flora. Europeans were not so adjusted and their arrival provided an impetus for increased productivity of domesticated species. Initial efforts were based on poorly adapted exotic crop plants in drought-prone and nutrient-poor soils. In effect, both genotype and environmental issues had to be addressed, but problems faced by early settlers were not articulated in those terms at that time.

Early selection of genetic variants better adapted to local conditions was mercifully successful, but largely empirical. Explanation and extrapolation into new environments had to wait for an emerging plant science to define underlying processes and adaptive responses — processes in the sense of genetically determined workings of plants that enabled them to be fitted them to a given niche in nature, adaptive responses in the sense that requirements for successful growth and reproductive development had to fit the environmental cues of a given location. Daylength, temperature and moisture stress proved to be important driving variables in this respect.

Development of plant science in Australia and New Zealand was shaped by issues of crop production, and a distinction in research philosophy gradually emerged between what might be termed process physiology and integrative plant science. Process physiology depends upon a reductionist approach to analysing the inner workings of plants at progressively finer

levels of organisation and contributes to our detailed 'understanding'. Such understanding finds application in both natural and managed ecosystems via integrative research.

Reductionists commonly use well-defined test systems with inherent properties that suit their analysis of particular processes such as ion uptake, photosynthetic electron flow or induction of flowering. They usually work within an established conceptual framework or paradigm, and define questions in a way that makes them amenable to test via experimentation with their test system. A well-defined system helps minimise ambiguity. Working paradigms do not remain fixed. Rather, they undergo often radical revision as new knowledge renders old paradigms untenable and a 'crisis' ensues (Kuhn 1970). A major revision of concepts pertaining to leaf gas exchange during the 1970s is a case in point. Discovery of C4 photosynthesis, definition of Rubisco function and an appreciation of photorespiration proved to be so highly congruent that a new paradigm emerged for gas exchange.

Whereas reductionist research implies an analysis of component parts at increasing levels of detail, integrative research implies a synthesis of interacting components to produce a model of plant function. Crop management models are built in this way, and cotton farming already depends closely on models used for decision-support systems. In that case, early attempts to account for variation in growth and reproductive development between seasons or across different locations were based on observation and inference. The resulting models 'worked', but were largely empirical and lacked a useful conceptual framework of component processes; they were of little use outside the reference frame within which they had been constructed. Current cotton models are process based, drawing upon an extensive knowledge of growth and developmental response to environmental inputs, especially water. As a result they are more generic and of wider predictive value.

A similar rationale applies to application of basic concepts in explaining adaptive features of any plant and, from that knowledge, predicting performance under defined conditions. Plantation forests are a case in point where likely scenarios of tree growth as a function of site quality need to be explored well ahead of investment decisions. Such analyses necessitate validated models which in turn draw upon comprehensive data sets of genotype × environment interactions on the physiology of particular tree species (e.g. see Battaglia and Sands (1997) for *Eucalyptus globulus*).

As an aside, when simulation models in agriculture, horticulture or forestry are used to apply outcomes from process physiology to real-world situations, significant gaps in basic understanding become apparent and can influence direction of future forest research. In this way, related streams of reductionist and integrative research also become interactive.

By analogy, *Plants in Action* seeks to engender such a two-way flow of information on all levels of organisation in vascular plants. Those levels stretch from gene expression during growth and reproductive development of individual plants to human and environmental selection pressures. Those unrelenting pressures shape genotypes and thus adaptation of wild species in nature, as well as the performance of domesticated plants in cultivation.

Further reading

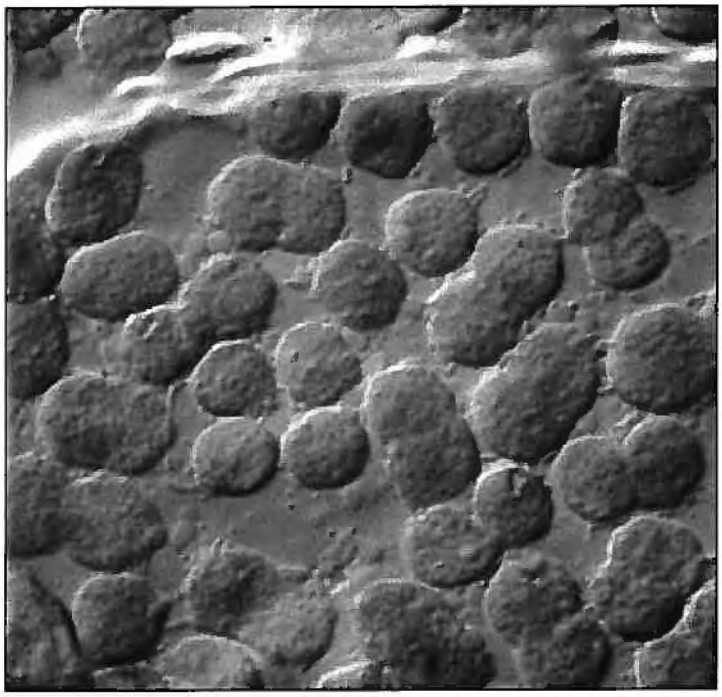
Evans, L.T. (1983). 'Science and the suburban spirit', *Search*, **13**, 307–311

Part II

Processes and resources for growth

Part II Contents

1 *Light use and leaf gas exchange*



Chloroplasts dividing (dumbell figures) within an enlarging cell of a young spinach leaf, resulting in about 200 chloroplasts per cell at leaf maturity (Nomarski optics)
(Light micrograph courtesy John Possingham)

...leaves seem also designed for many other noble and important services, plants very probably drawing thro' their leaves some part of their nourishment from the air. May not light also, by freely entering the expanded surfaces of leaves and flowers, contribute much to the enobling the principles of vegetables?...

(Stephen Hales, 'Vegetable Staticks' 1727)

Chapter outline

Introduction

Leaves epitomise adaptive responses in vascular plants where genotype × environment interactions impact on both form and function. Endless variations in leaf size, shape and pose attest adaptation in form, while qualitative differences in photosynthetic mode reflect contrasting function.

Despite such variation, leaves fulfil a common purpose: to capture energy from sunlight and convert that currency into chemically useful forms to drive CO_2 assimilation and subsequent growth. Light absorption and energy utilisation is considered at progressively finer levels of organisation from leaves (Section 1.1) to chloroplasts (Section 1.2).

Section 1.1 encompasses anatomy, light interception and leaf gas exchange and includes a case study on development of a process-based model for photosynthetic CO_2 assimilation using A:p_i curves.

1.1 Leaf anatomy, light interception and gas exchange

Leaves have evolved into a myriad of sizes and shapes, showing great variation in surface features and internal anatomy. Nevertheless, these organs all share a common function, namely to intercept sunlight and facilitate CO_2 uptake while restricting water loss. The wide variety of shapes, sizes and internal structure that leaves display implies that many solutions exist to meet the mixed demands of leaf function under frequently adverse conditions.

In nature, photon irradiance (photon flux density) can fluctuate over three orders of magnitude and these changes can be rapid. However, plants have evolved with photosynthetic systems that operate most efficiently at low light. Such efficiency confers an obvious selective advantage under light limitation, but predisposes to photodamage under strong light. How then can leaves cope? First, some tolerance is achieved by distributing light over a large population of chloroplasts held in architectural arrays within mesophyll tissues. Second, each chloroplast can operate as a seemingly independent entity with respect to photochemistry and biochemistry and can vary allocation of resources between photon capture and capacity for CO_2 assimilation in response to light climate. Such capacities confer great flexibility across a wide range of light environments where plants occur and are discussed in Chapter 13.

Photon absorption is astonishingly fast (single events lasting 10^{-15} s). Subsequent energy transduction into NADPH and ATP is relatively 'slow' (10^{-4} s), and is followed by CO_2 fixation via Rubisco at a sedate pace of 3.5 events per second per active site, and is generally constrained by even slower diffusion processes. Distributing light absorption between many chloroplasts thus equalises effort over a huge population of these organelles, but also reduces diffusion limitations by allowing placement of chloroplasts at optimal locations within each cell. The internal structure of leaves (Figures 1.1 and 1.3) reflects this need to maximise CO_2 exchange between intercellular airspace and chloroplasts and to distribute light more uniformly with depth than would occur in an homogeneous solution of chlorophylls.

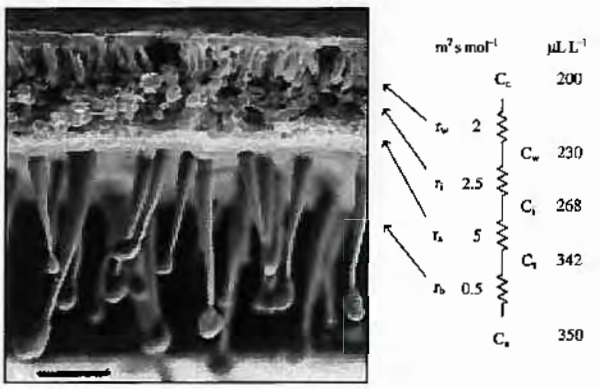


Figure 1.1 A scanning electron micrograph of an uncoated and rapidly frozen piece of tobacco leaf showing a hairy lower leaf surface and cross-sectional anatomy at low magnification. Epidermal outgrowths (hairs) offer some protection against insects, and contribute to formation of a boundary layer (unstirred air) adjacent to lower leaf surfaces. An electrical analogue (right side) shows a series of resistances (r) that would be experienced by CO_2 molecules diffusing from outside (ambient) air to fixation sites inside chloroplasts. Subscript 'b' refers to boundary layer, 's' to stomatal, 'i' to intercellular airspaces, 'w' to cell wall and liquid phase. Notional values for these resistances are given in units of m^2 s mol^{-1}, and emphasise the prominence of stomatal resistance within this series. Corresponding values for CO_2 concentration are shown in µL L^{-1}, and reflect photosynthetic assimilation within leaves generating a gradient for inward diffusion. In that case, subscript 'a' refers to ambient air, 's' to leaf surface, 'i' to substomatal cavity, 'w' to mesophyll cell wall surface, 'c' to sites of carboxylation within chloroplasts. c_i is routinely inferred from gas exchange measurements and used to construct A:c_i curves for leaf photosynthesis (cf. A:p_i curves in Figure 1.11). Horizontal bar lower left is 100 µm

(Original illustration from Jian-Wei Yu and John Evans, unpublished)

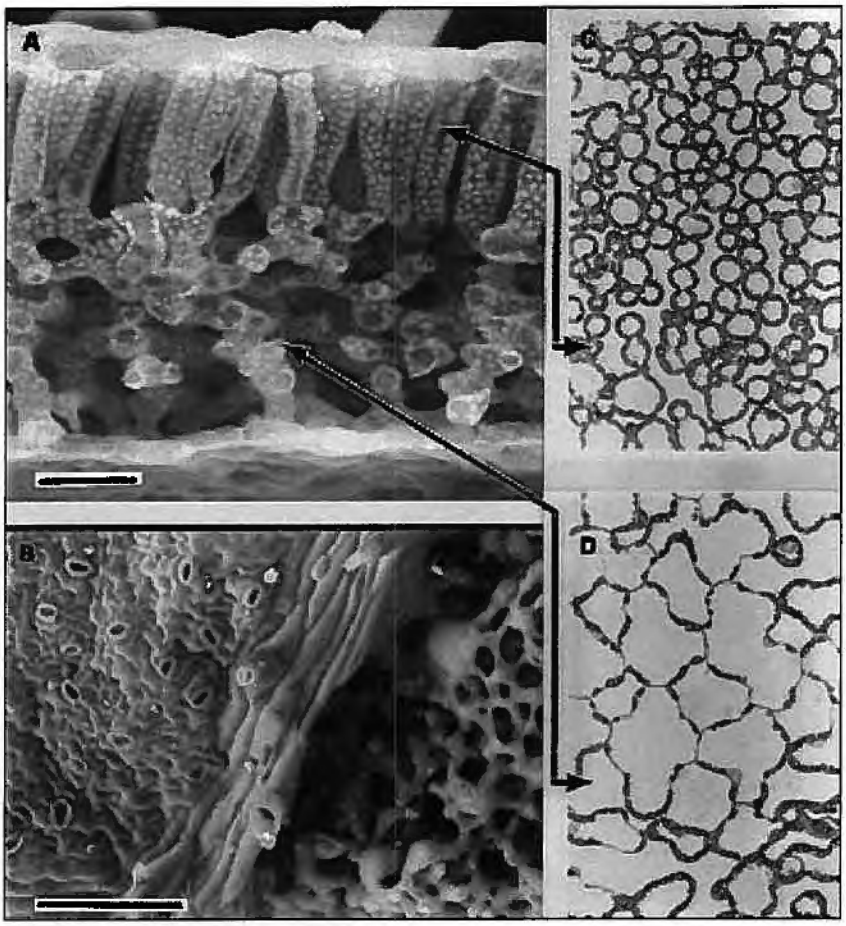


Figure 1.2 A scanning electron micrograph of an uncoated and rapidly frozen piece of tobacco leaf fractured in (A) to reveal palisade mesophyll cells beneath the upper leaf surface and spongy mesophyll in the lower half. Chloroplasts can be clearly seen covering the inner faces of cell walls. Looking onto the lower surface (B), the epidermis and stomata are present on the left side of the vein, whereas the epidermis was fractured away on the right side, revealing spongy mesophyll tissue. Light micrographs (C,D) of sections cut parallel to the leaf surface are shown for palisade (C) and spongy mesophyll (D) with solid lines showing where the paradermal sections align with (A). Chloroplasts form a dense single layer covering the cell surfaces exposed to intercellular airspace, but are rarely present lining walls where two cells meet. Horizontal bar in (A) is 50 μm and in (B) is 200 mm. Magnification given in (A) also applies to (C) and (D)
(Original illustration from John Evans and Susanne von Caemmerer. See Evans *et al.* (1994) for related material)

1.1.1 Leaf structure

In a typical herbaceous dicot (Figure 1.1) lower leaf surfaces are covered with epidermal outgrowths, known to impede movement of small insects, but also contributing to formation of a boundary layer. This unstirred zone immediately adjacent to upper and lower epidermes varies in thickness according to surface relief, area and wind speed. Boundary layers are significant in leaf heat budgets and feature in the calculation of stomatal and mesophyll conductances from measurements of leaf gas exchange.

In transverse fracture (Fignre 1.2A) the bifacial nature of leaf mesophyll is apparent with columnar palisade cells beneath the upper surface and irregular shaped cells forming the spongy mesophyll below. Large intercellular airspaces, particularly in the spongy mesophyll, facilitate gaseous diffusion. The lower surface of this leaf is shown in Figure 1.2B. On the left-hand side, the epidermis is present with its irregular array of stomata. Diagonally through the centre is a vein with broken-off hair cells and on the right, the epidermis has been fractured off revealing spongy mesophyll cells. Light micrographs of sections cut parallel to the leaf surface (paradermal) through palisade (C) and spongy (D) tissue reveal chloroplasts lying in a single layer and covering most of the internal cell wall surface adjacent to airspaces. Significantly, they are rarely present on walls that adjoin another cell. Despite the appearance of close packing, palisade cell surfaces are generally exposed to intercellular airspace. Inward diffusion of CO_2 to chloroplasts is thereby facilitated.

Leaves that develop in sunny environments and have high photosynthetic capacities are generally thicker than leaves

from shaded environments. This is achieved with more elongate palisade cells and/or several layers of palisade cells. Thicker leaves in a sunny environment prove energy effective because enough photons reach chloroplasts in lower cell layers to keep their Rubisco gainfully employed. Such depth deploys sufficient Rubisco to confer a high photosynthetic capacity. By contrast, in a shaded habitat, less Rubisco is required for a leaf with lower photosynthetic capacity, and this can be achieved with thinner leaves.

1.1.2 Light absorption

Pigments in thylakoid membranes of individual chloroplasts (Figure 1.7) are ultimately responsible for strong absorption of wavelengths corresponding to blue and red regions of the visible spectrum (Figure 1.3). Irradiated with red or blue light, leaves appear dark due to this strong absorption, but in white light leaves appear green due to weak absorption around 550 nm, which corresponds to green light. Ultraviolet (UV) light (wavelengths below 400 nm) can be damaging to macromolecules, and sensitive photosynthetic membranes also suffer. Consequently, plants adapt by developing an effective sunscreen in their cuticular and epidermal layers.

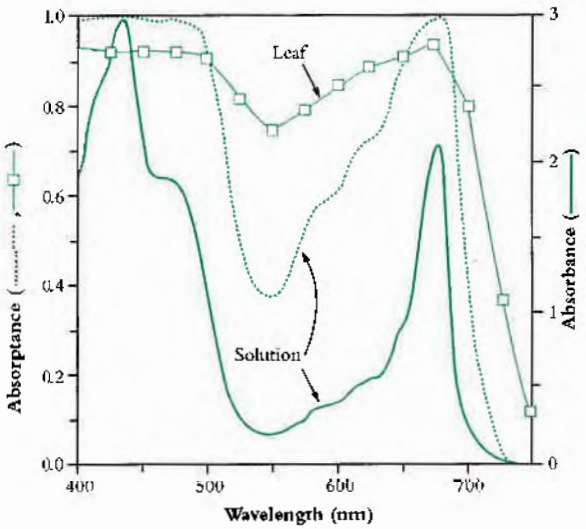


Figure 1.3 Light absorption by pigments in solution and by leaves. Absorbance (A) refers to attenuation of light transmitted through a leaf or a solution of leaf pigments, as measured in a spectophotometer, and is derived from the expression $A = \log I_0/I$ where I_0 is incident light, and I is transmitted light. The solid curve (scale on right ordinate) shows absorbance of a solution of pigment–protein complexes equivalent to that of a leaf with 0.5 mmol Chl m^{-2}. The dotted curve shows absorptance (scale on left ordinate), and represents the fraction of light entering the solution that is absorbed. Virtually all light between 400–500 nm and around 675 nm is absorbed, compared with only 40% of light around 550 nm (green). The dashed curve with squares represents leaf absorptance, which does not reach 1 because the leaf surface reflects part of the incident light. Of light around 550 nm, 75% is absorbed because leaves scatter light effectively which increases the path length and thereby increases probability of absorption above that observed for the same pigment concentration in solution
(Replotted data from McCree 1972; Evans and Anderson 1987)

Overall, absorption of visible light by mesophyll tissue is complex due to sieve-effects and scattering. Sieve-effect is an outcome from packaging pigments into discrete units, in this case chloroplasts, while remaining leaf tissue is transparent. This increases the probability that light can bypass some pigment and penetrate more deeply. A regular, parallel arrangement of palisade cells with chloroplasts all vertically aligned means that about 80% of light entering a leaf initially bypasses the chloroplasts, and measurements of absorption in an Ulbright sphere confirm this. Scattering occurs by reflection and refraction of light at cell walls due to the different refractive indices of air and water. Irregular-shaped cells in spongy tissues enhance scattering, increasing the path length of light travelling through a leaf and thus increasing the probability of absorption. Path lengthening is particularly important for those wavelengths more weakly absorbed and results in at least 80% absorption, even at 550 nm (Figure 1.3). Consequently, leaves typically absorb about 85% of incident light between 400 and 700 nm; only about 10% is reflected and the remaining 5% is transmitted. These percentages do of course vary according to gene × environmental factors, and especially adaptation to aridity and light climate.

Sunlight entering leaves is attenuated with depth in much the same way as light entering a canopy of leaves shows a logarithmic attenuation with depth that follows Beer's law (Section 0.0). Within individual leaves, the pattern of light absorption is a function of both cell anatomy and distribution of pigments. An example of several spatial profiles for a spinach leaf is shown in Figure 1.4. Chlorophyll density peaks in the lower palisade layer and decreases towards each surface, declining exponentially with cumulative chlorophyll. Light absorption is then given by the product of the chlorophyll and light profiles. This increases from the upper surface, peaking near the base of the first palisade layer, then declines steadily towards the lower surface. Because light is the pre-eminent driving variable for photosynthesis, CO_2 fixation tends to follow the light absorption profile (see ^{14}C fixation pattern in Figure 1.4). However, the profile is skewed towards the lower surface because of a non-uniform distribution of photosynthetic capacity. Chloroplasts near the upper surface have 'sun'-type characteristics which include a higher ratio of Rubisco to chlorophyll and higher rate of electron transport per unit chlorophyll. Chloroplasts near the lower surface show the converse features of 'shade' chloroplasts. Similar differences between 'sun' and 'shade' leaves are also apparent. Chloroplast properties do not change as much as the rate of absorption of light. Consequently, the amount of CO_2 fixed per quanta absorbed increases with increasing depth beneath the upper leaf surface. The lower half of a leaf absorbs about 25% of incoming light, but is responsible for about 31% of a leaf's total CO_2 assimilation.

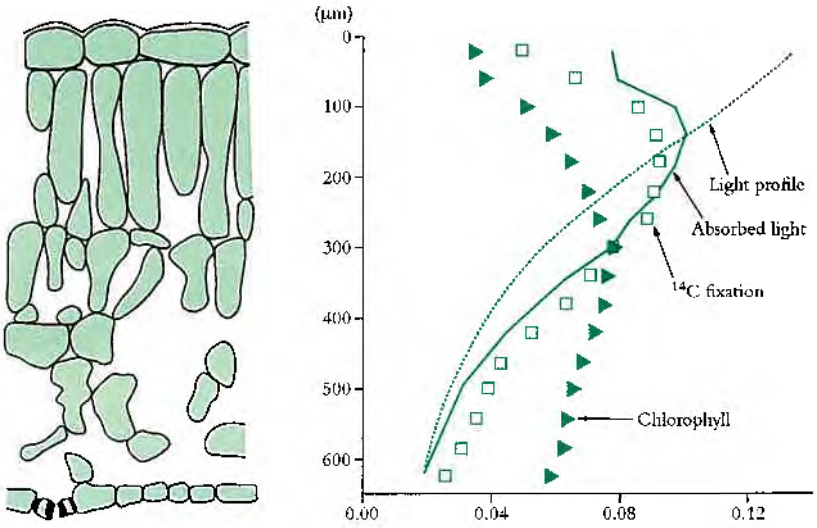


Figure 1.4 Profiles of chlorophyll, light absorption and photosynthetic activity through a spinach leaf. Cell outlines are shown in transverse section (left side). Triangles represent the fraction of total leaf chlorophyll in each layer. The light profile (dotted curve) can then be calculated from the Beer–Lambert law. The profile of absorbed light is thus the product of the chlorophyll and light profiles (solid curve). CO_2 fixation, revealed by ^{14}C labelling, follows the absorbed light profile, being skewed towards slightly greater depths
(Replotted data from Nishio *et al.* 1993; Evans 1995)

1.1.3 CO_2 diffusion to chloroplasts

Leaves are covered with a barrier or 'cuticle' on the outer walls of epidermal cells that is impermeable to both water and CO_2. Accordingly, CO_2 used in leaf photosynthesis gains entry via stomata (Figure 1.5), and as CO_2 molecules diffuse inwards they encounter an opposite flux, 3–4 orders of magnitude stronger, of H_2O molecules rushing outwards. Leaves control this gas exchange by adjusting the aperture of stomata which can vary within minutes in response to changes in several environmental variables including light, humidity and CO_2 concentration (see Chapter 16 for more details). Air spaces inside leaves are effectively saturated with water vapour (equivalent to 100% relative humidity at that leaf temperature) and because air surrounding illuminated leaves is almost universally drier, water molecules diffuse down this concentration difference from leaf to air.

The diffusion pathway for H_2O is usually divided into two parts, namely the boundary layer of still air at the leaf surface and stomatal pores (Figure 1.5). Boundary layer thickness depends on windspeed, leaf dimensions and the presence of surface structures (e.g. hairs in Figure 1.1). Positioning of stomata also varies between species. Leaves of terrestrial plants always have stomata on their lower (abaxial) surface but many species have stomata on both surfaces, especially if they have high photosynthetic rates and are in sunny locations such as pendulant leaves of eucalypts. Adaptations for arid environments include having surface structures like hairs and waxes, which increase the thickness of the boundary layer, and leaf

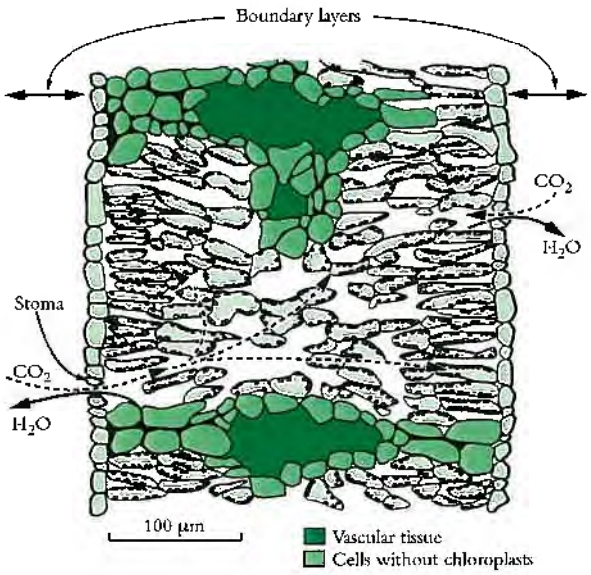


Figure 1.5 Diagram of a transverse section through a bilateral *Eucalyptus pauciflora* leaf which is normally pendulant. Palisade tissue occurs beneath both surfaces with spongy tissue and oil glands (not shown) in the middle. A putative pathway for diffusion of H_2O out of the substomatal cavity is shown by the solid curved arrows while CO_2 entry is represented by dashed curved arrows. CO_2 diffuses inwards and H_2O diffuses outwards in response to leaf–air concentration differences. Such gas exchange is restricted by a boundary layer (the unstirred layer of air at the leaf surface) and by stomata. One stoma is shown on each surface. CO_2 diffusion continues inside the leaf mesophyll through airspaces between cells (curved dashed arrows) to reach cell walls adjacent to each chloroplast where CO_2 dissolves and then diffuses into the chloroplast to reach the carboxylating enzyme Rubisco. Bundle sheath extensions (right side of diagram) reach both epidermes and create an internal barrier to lateral diffusion
(Adapted from Evans *et al.* 1993)

rolling and encryption of stomata by placing them in crevices in the leaf surface. While these features restrict water loss, they also impose an increased resistance (decreased conductance) to CO_2 uptake.

CO_2 molecules diffusing inwards from ambient air to chloroplasts encounter restrictions additional to boundary layer and stomata (Figure 1.5). CO_2 must also diffuse from substomatal cavities throughout the mesophyll, dissolve in wet cell walls, enter the cytosol across a plasmalemma, diffuse into chloroplasts across a double membrane (outer envelope in Figure 1.9) and finally reach fixation sites within the stroma of those chloroplasts.

There is considerable variation in leaf anatomy and hence potential restriction to CO_2 diffusion, but in general leaves with high rates of photosynthesis tend to have more permeable leaves (e.g. tobacco in Figure 1.2) and this complex anatomy ensures a greatly enlarged surface area for diffusion across interfaces. Indeed the total mesophyll cell wall area can be 20 times that of the projected leaf surface.

Diffusion to chloroplasts is further enhanced by their tendency to appress in clusters against cell walls adjacent to intercellular spaces (Figure 1.2 C,D), while carbonic anhydrase within chloroplasts speeds up diffusion of CO_2 by catalysing interconverion of CO_2 and bicarbonate within the stroma of chloroplasts. Although CO_2 rather than HCO_3^-, is the substrate species for Rubisco, the presence of carbonic anhydrase enables bicarbonate ions, more abnndant under the alkaline conditions (pH 8.0) that prevail inside chloroplasts, to diffuse to Rubisco in concert with diffusion of CO_2. By sustaining a very rapid equilibration between CO_2 and HCO_3^- immdediately adjacent to active sites on Rubisco, carbonic anhydrase enhances inward diffusion of inorganic carbon.

1.1.4 Light and CO_2 effects on leaf photosynthesis

Light impinging on plants arrives as discrete particles we term photons, so that a flux of photosynthetically active photons can be referred to as 'photon irradiance'. A quantum (plural quanta) refers to a parcel of light energy carried by a photon, and is used here when referring to photosynthetic utilisation of energy derived from absorbed photons as in 'quantum efficiency'. Physicists recognise this subtle difference between 'photons' and 'quanta' and accord correct use. Plant biologists (incorrectly) use them interchangeably, a convention followed here.

CO_2 assimilation varies according to both light and CO_2 partial pressure. At low light (low photon irradiance in Figure 1.6) assimilation rate increases linearly with increasing irradiance, and the slope of this initial response represents maximum qnantum yield (mol CO2 fixed per mol quanta absorbed). Reference to *absorbed* quanta in this expression is important. Leaves vary widely in surface characterisnics (hence reflectance) as well as internal anatomy and chlorophyll concentration (Chlorophyll content per unit mesophyll volume). Therefore absorption of photosynthetically active quanta will vary, so that quantum yield expressed in terms of incident irradiance can be misleading, and in the case of comparisons between sun and shade leaves has led to a widely held hut mistaken belief that shade leaves (thinner and with higher chlorophll concentration) are more efficient. Expressed in terms of absorbed quanta, sun and shade leaves have virtually identical quantum efficiencies for CO_2 assimilation.

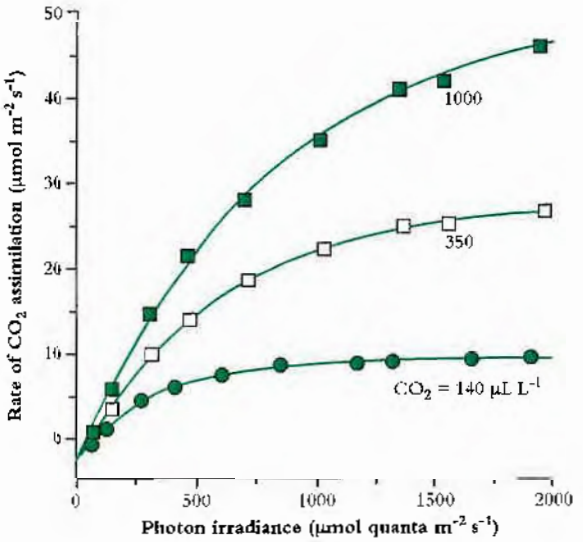


Figure 1.6 Photosynthetic response to photon irradiance for a *Eucalyptus maculata* leaf measured at three ambient CO_2 concentrations, 140, 350 and 1000 μmol mol^{-1}. Irradiance is expressed as μmol quanta of photosynthetically active radiation absorbed, and net CO_2 assimilation is inferred from a drop in CO_2 concentration of gas passing over a leaf held in a temperature-controlled cuvette. CO_2 evolution in darkness is shown on the ordinate as an extrapolation below zero. The irradiance at which net CO_2 exchange is zero equals the light compensation point (commonly 15–50 μmol quanta m^{-2} s^{-1}, shade to sun species respectively). The initial slope of light-response curves for CO_2 assimilation per absorbed qnanta represents maximum quantum yield for a leaf
(Adapted from Ögren and Evans 1993)

Assimilation rate increases more slowly at higher irradiances until eventually a plateau is reached where further increases in irradiance do not increase the rate of CO_2 assimilation (Figure 1.6). Chloroplasts are then light saturated. Absolute values for both quantum yield and light-saturated plateau depend on CO_2 partial pressure. Quantum yield increases as CO_2 partial pressure increases because photorespiration is progressively suppressed. At ambient CO_2, photorespiration normally consnmes about one-third of available photochemical energy. The qnantum yield for incident light also depends primarily on chlorophyll content. Leaf absorptance has a hyperbolic dependence on chlorophyll content. For most leaves, 80–85% of 400–700 nm light is absorbed and it is only in leaves produced under severe nitrogen deficiency which have less than 0.25 mmol Chl m^{-2} that absorptance falls below 75%.

Case study 1.1 Development of A:p_i curves

By Susanne von Caemmerer and Graham Farquhar

Component processes underlying CO_2 assimilation carry both biophysical and biochemical dimensions but are nevertheless amenable to analysis at a whole-leaf level. Using continuous flow gas analysers, CO_2 exchange in both light and dark is easily measured, and component processes inferred. Under strong illumination, CO_2-assimilation clearly predominates over CO_2-generating processes (both mitochondrial and photorespiration), and in those circumstances the inward flux of CO_2 can be taken as a net reaction rate for CO_2 assimilation via Rubisco as primary catalyst. However, if whole-leaf photosynthesis is to be analysed in biochemical terms, the effective concentration of this primary substrate at fixation sites must also be known. How then can these substrate levels be defined in an actively photosynthesising leaf? Moreover, knowing that CO_2 assimilation is energy dependent, and that both ATP and reducing power (NADPH) are being generated concurrently, how can photosynthetic electron flow be described in terms relevant to CO_2 assimilation? A conjunction between biochemistry and photobiophysics was clearly required, and a paradigm shift away from resistance analogues of leaf gas exchange was necessary for biologically meaningful models of leaf photosynthesis. This case study traces those developments.

Early models of leaf gas exchange had been developed as electrical analogues of resistances, and proved useful in making a distinction between stomatal and mesophyll limitations on CO_2 assimilation. Mesophyll, or 'residual', resistance was a collective term that was meant to embody non-stomatal diffusive factors, and included both physical and biochemical constraints. Further refinement would depend upon a reliable estimate of CO_2 partial pressure at fixation sites within leaves, and those estimates came with improvements in diffusive models for leaves, but, in particular, development of high- precision gas exchange systems with a capacity for fast data analysis (either by interfacing measuring devices with computers, or via chart recorder and human agency!). CO_2 response curves emerged as a valuable tool to analyse photosynthesis *in vivo*.

Physical concepts of leaf gas exchange

Penman and Schofield (1951) put diffusion of CO_2 and water vapour through stomata on a firm physical basis. Their ideas were taken up at Wageningen by Pieter Gaastra in the 1950s and modern analytical gas exchange is often attributed to this seminal work (Gaastra 1959) where he even constructed his own infrared gas analyser and other equipment necessary to make measurements of CO_2 and water vapour exchange. His work was a landmark because it examined CO_2 assimilation and water vapour exchange rates of individual leaves under different environmental conditions, and he distinguished between stomatal and internal resistances. Gaastra calculated resistances to water vapour and CO_2 diffusion from two equations (here in our simplified notation) which are based on Fick's Law for the diffusion of gases.

$$E = \frac{w_i - w_a}{r_{sw}} \text{ and } A = \frac{c_a - c_i}{r_{sc}} \qquad (1)$$

where E and A are the floxes of water vapour and CO_2 and w_i and c_i and w_a and c_a are the mole fractions of water vapour and CO_2 in intercellular air spaces and ambient air respectively. Denominator terms r_{sw} and r_{sc} represent stomatal resistances to H_2O and CO_2 diffusion respectively. Gaastra calculated w_i from the saturated vapour pressure at the measured leaf temperature and since both E and w_a were measured variables this allowed r_{sw} to be calculated. Knowing that resistances to CO_2 and water vapour are related by the ratio of their diffusivities, he calculated stomatal resistance to CO_2 diffusion, r_{sc}. Gaastra realised that the diffusion path for CO_2 is longer than that of water vapour, as CO_2 had to diffuse from the intercellular airspaces through the cell wall across membranes to the chloroplast stroma where CO_2 fixation by Rubisco takes place. He therefore extended the equation for CO_2 assimilation to:

$$A = \frac{c_a - c_{chl}}{r_{sc} + r_{mc}} \qquad (2)$$

Where c_{chl} represented CO_2 concentration at chloroplasts.

Gaastra analysed the dependence of CO_2 assimilation rate on light, CO_2 and temperature, and observed that at low CO_2 concentrations the rate of CO_2 assimilation was independent of temperature whereas it was strongly influenced by temperature at higher CO_2 concentrations. This led him to conclude that the rate of CO_2 uptake was completely limited by CO_2 diffusion processes at low CO_2 and that biochemical processes became limiting only at high CO_2. The belief that CO_2 diffusion was limiting led him to assume that the chloroplastic CO_2 concentration was close to zero. It allowed a welcome simplification of the above equation such that the total resistance to CO_2 diffusion could be calculated from CO_2 assimilation rate and the ambient CO_2 concentration alone. Since stomatal resistances could be calculated from measurements of water vapour diffusion, it was also possible to calculate mesophyll resistance to CO_2 diffusion. In Australia particularly there was a great interest in determining the relative importance of stomatal and mesophyll resistance in limiting CO_2 assimilation rates under adverse conditions of high temperature and frequent water stresses, and in global terms much of the pioneering work was undertaken in this country (see, for example, Bierhuizen and Slatyer 1964).

Calculation of intercellular CO_2, c_i and the first A v.s c_i curves

Although CO_2 concentration in intercellular air spaces, c_i, was explicit in Gaastra's equations, this term was first specifically calculated by Moss and Rawlings in 1963, and the first extensive nse of the parameter was made by Whiteman and Koller in 1967, who examined stomatal responses to CO_2 and irradiance, concluding that stomata were more likely to respond to c_i rather than c_a. The first *bona fide* response curves of CO_2 assimilation rate to c_i rather than c_a were those of Troughton (1969) and Troughton and Slatyer (1969) (Figure 1). In Figure 1(a), c_i was derived from measurements of CO_2 uptake in an assimilation chamber where air passed through a leaf, rather than over both surfaces concurrently (as became commonplace in subsequent designs), and such estimates would differ slightly. More importantly, those measurements were made at different temperatures and confirmed that CO_2 assimilation was not greatly affected by temperature at low c_i. Later, this lack of temperature dependence was explained by the kinetics of Rubisco (von Caemmerer and Farquhar 1981). Figure 1(b) shows the initial slope of CO_2 response curves measured at different stages of water stress. In this case, water stress has affected stomatal resistance (as the c_i obtained at air levels of CO_2 occur at progressively lower c_i) but not the relationship between CO_2 assimilation rate and c_i. A versus c_i response curves thus provided an unambiguous distinction between stomatal and non-stomatal effects on CO_2 assimilation and, provided stomata respond uniformly across both leaf surfaces, that distinction can be made quantitative.

Before we head further into a discussion of our 1990s understanding and interpretation of more comprehensive CO_2 response curves, we must take an important digression into development of mathematical models of C_3 photosynthesis.

Biochemistry of photosynthesis and leaf models

Gas exchange studies focused initially on physical limitations to diffusion, but it was not long before persuasive arguments were being brought forward to show that leaf biochemistry must influence the rate of CO_2 fixation even at low CO_2 concentrations. Björkman and Holmgren (1963) made careful gas exchange measurements of sun and shade ecotypes of Solidago growing in Sweden, and noted strong correlations between photosynthetic rate measured at high irradiance and ambient CO_2 and the nitrogen content of leaves, and later also related it to different concentrations of Rubisco (then called carboxydismutase). Anatomical studies implied that thin shade leaves would not have larger internal diffusion resistance to CO_2 than thicker sun leaves where cells were more densely packed. Furthermore, following earlier discoveries of the O_2 sensitivity of photosynthesis, namely a low-O_2 enhancement of CO_2 assimilation rate, Gauhl and Björkman (1969), then at Stanford, showed very elegantly that while O_2 concentration did affect CO_2 assimilation rate, water vapour exchange was not affected (i.e. stomata had not responded). Clearly, the increase in CO_2 assimilation rates seen with a decrease in O_2 concentration could not be explained via a limitation on CO_2 diffusion.

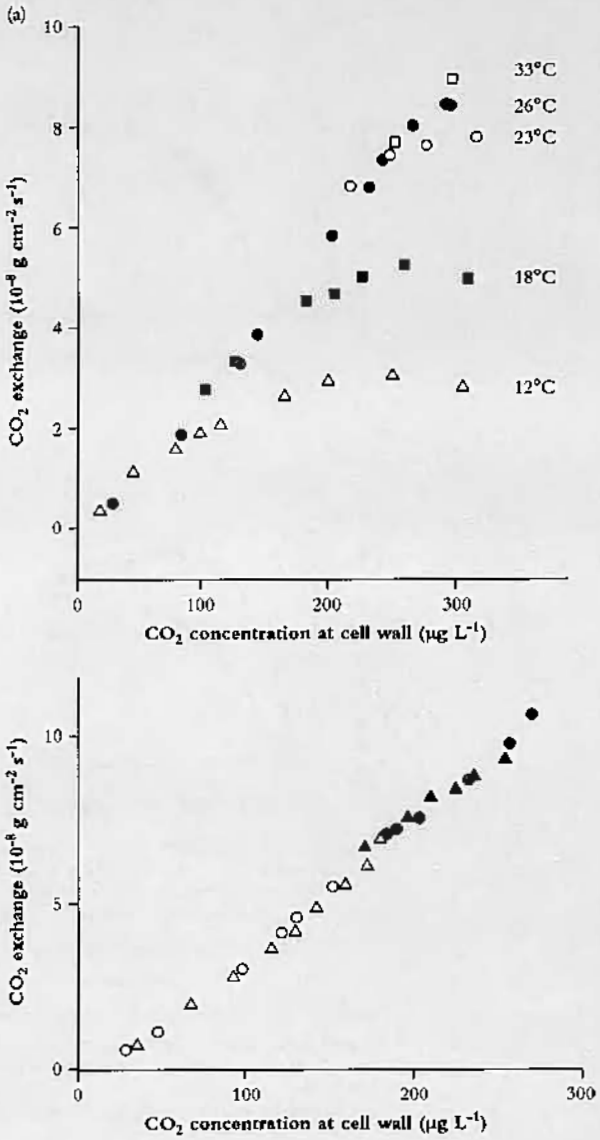


Figure 1 An early A:p_i curve showing the CO_2 assimilation rate of cotton at a range of cell wall CO_2 concentrations (redrawn from Troughton (1969) and Troughton and Slatyer (1969) and retaining original units for CO_2 flux). For comparative purposes, 10×10^{-8} g. cm^{-2} s^{-1} would be equivalent to 22.27 μmol CO_2 m^{-2} s^{-1}, and 1 μg l^{-1} would be equivalent to 0.54 ppm (assuming a gram molecular weight of 44 for CO_2, and measurements at normal temperature and pressure). (a) Leaf temperature influences the overall shape of CO_2 response curves (measured in O_2-free air) but has no effect on the initial slope where response to CO_2 is limited by Rubisco activity family of curves comes from repeated measurements of gas exchange by the same leaf at five different temperatures (values shown) and indicated in the figure by five different symbols. (b) CO_2 response curves for two leaves of cotton measured in O_2-free air at 25°C and three levels of relative water content. Legend: ● leaf 1, 92% water content; ○ leaf 1, 56%; ▲ leaf 2, 92%; △ leaf 2, 69%. Identical slopes regardless of treatment mean that variation in relative water content over this range is without effect on CO_2 assimilation within mesophyll tissues. By implication, reduction in CO_2 uptake as commonly observed on whole leaves under moisture stress would be attributable to stomatal factors

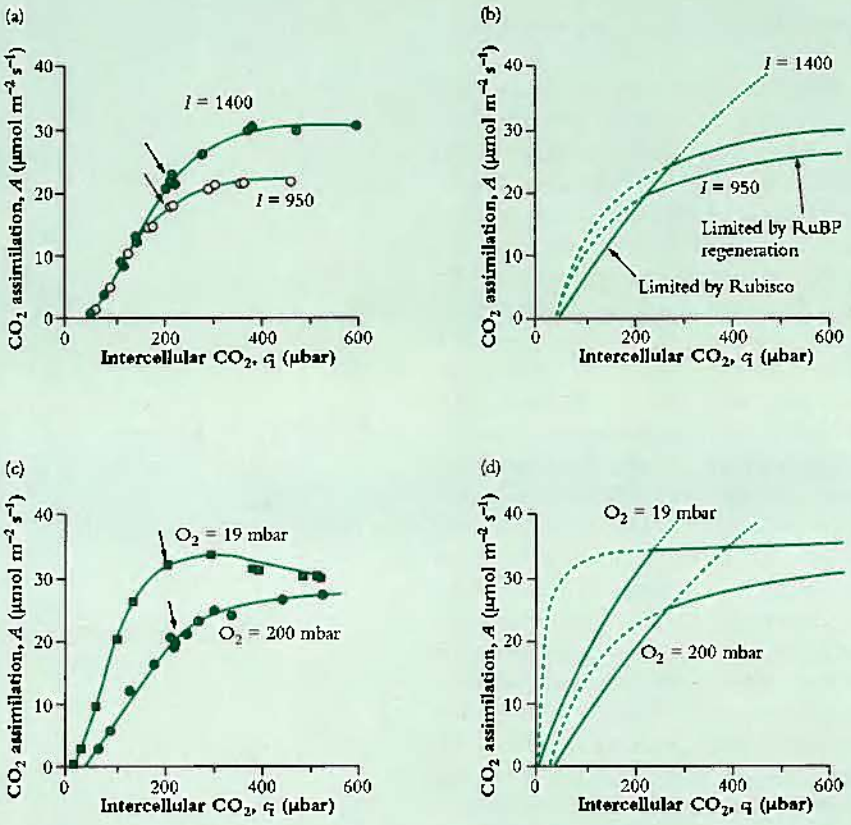


Figure 2 Comparison of measured and modelled CO_2 reponse curves. (a) CO_2 assimilation rate v. intercellular CO_2 concentration in *Phaseolus vulgaris* measured at two irradiances and a leaf temperature of 28°C. Arrows indicate points obtained at an external CO_2 concentration of 330 μbar. (b) Modelled CO_2 response curves. The dotted line and its extension represent the Rubisco-limted rate of CO_2 assimilation ($A = \frac{(c_i - \Gamma_*)V_{cmax}}{(c_i + K_c(1 + O/K_o)} - R_d$). The dashed lines and their extensions represent the electron-transport-limited rates of CO_2 assimilation at the two irradiances ($A = \frac{(c_i - \Gamma_*)J}{4.5c_i + 10.3\Gamma_*} - R_d$). For further details see von Caemmerer and Farquhar (1981). (c) CO_2 assimilation rate v. intercellular CO_2 concentration in *Phaseolus vulgaris* measured at two O_2 concentrations at a leaf temperature of 28°C. Arrows indicate points obtained at an external CO_2 concentration of 330 μbar. (d) Modelled CO_2 response curves for conditions applied in (c). (Method details in (b))

Central importance of Rubisco

Early mathematical models of leaf photosynthesis were extensions of Gaastra's resistance equation, and could not accommodate the O_2 sensitivity of CO_2 assimilation. They were quickly followed by development of more biochemical models in the early 1970s and the discoveries by Bowes *et al.* (1971) that Rubisco was responsible for both carboxylation and oxygenation of RuBP (a five-carbon phosphorylated sugar, regenerated by the PCR cycle of chloroplasts). This crucial observation of dual function put Rubisco at centre stage. Laing *et al* (1974) were first to compare the gas exchange of soybean leaves with the *in vitro* kinetics of Rubisco and suggested the following equation for the net CO_2 assimilation rate:

$$A = V_c\left(1 - 0.5\frac{V_o}{V_c}\right) \quad (3)$$

where V_c and V_o are the rates of Rubisco carboxylation and oxygenation (later on a term for mitochondrial respiration was added to most models). Laing *et al.* related a ratio of the rates of carboxylation to oxygenation of RuBP to the concentration of its substrates, CO_2, C, and O_2, O, and showed that:

$$\frac{V_o}{V_c} = \frac{V_{omax}K_c}{V_{cmax}K_o}\frac{O}{C} = 2\Gamma\star/C \quad (4)$$

where K_c, K_o, V_{cmax}, V_{omax} are the corresponding Michaelis

Menten constants and maximal activities of carboxylase and oxygenase functions respectively and G* is the CO_2-compensation point in the absence of mitochondrial respiration.

A note on Γ: illuminated leaves held in a closed circuit of recirculating air will reduce CO_2 to a 'compensation point' where uptake and generation of CO_2 are balanced, this is commonly 50–100 ppm for C_3 plants and referred to as Γ. A CO_2-response curve for leaf photosynthesis will show a similar value as an intercept on the abscissa. Γ can thus be measured empirically, and will be an outcome of interactions between photosynthesis, photorespiration and dark (mitochondrial) respiration (R_d). If allowance is made for R_d, the CO_2 compensation point would then be slightly lower, and is termed Γ*. As with measured Γ, this inferred CO_2 compensation point, Γ*, is linearly related to O_2, an observation that intrigued earlier observers but was easily reconciled with the dual function of Rubisco. Laing *et al.* (1974) used Equations 3 and 4 to predict this linear dependence of Γ* on O_2, and with subsequent confirmation Rubisco became a key player in photosynthetic models. (Equation 4 assumes that for each oxygenation, 0.5 CO_2 are evolved in the subsequent photorespiratory cycle, although there has been some debate over this stoichiometry.) If the enzyme reaction is ordered with RuBP binding first, the rate of carboxylation in the presence of the competitive inhibition by CO_2 at saturating RuBP concentration can be given by

$$V_c = \frac{CV_{cmax}}{C + K_c(1 + O/K_o)} \qquad (5)$$

When combined with the previous expression this gave a simple expression of net CO_2 fixation rate:

$$A = \frac{(c_i - \Gamma^*)V_{cmax}}{(c_i + K_c(1 + O/K_o))} \qquad (6)$$

which depends on the maximal Rubisco activity and provided the quantitative framework for comparing rates of CO_2 assimilations with the amount of Rubisco present in leaves (von Caemmerer and Farquhar 1981). Difference in CO_2 assimilation rates observed under different growth conditions could then be explained according to variations in the amount of Rubisco present in leaves. In Figure 2 the dotted line shows a CO_2 response curve modelled by Equation 6. Chloroplast CO_2 partial pressure was then assumed to be similar to that in the intercellular air spaces. Using on-line discrimination between $^{13}CO_2$ and $^{12}CO_2$, and deriving an estimate of CO_2 partial pressure at fixation sites within chloroplasts, we subsequently learned that a further draw down can occur, but the general applicability of Equation 6 was not compromised. As an aside, these equations became basic to most photosynthetic models long before the order of the reaction mechanism of Rubisco had been unequivocally established. Had CO_2 and O_2 bound to Rubisco before RuBP, or the reaction not been ordered, our equations would have been much more complex with both $K_m(CO_2)$ and $K_m(O_2)$ dependent upon RuBP concentration.

Regeneration of RuBP and electron transport rate

Equation 6 could mimic CO_2 assimilation rate at low c_i, as well as O_2 effects on CO_2 uptake, but measured rates of CO_2 assimilation saturated much more abruptly at high CO_2 concentrations than could be predicted from Rubisco kinetics (Figure 2). Using a highly novel approach in Estonia, Laisk and Oja (1974) proposed that CO_2 assimilation was limited by RuBP regeneration rate at high c_i. They had fed brief pulses of CO_2 to leaves that had been previously exposed to low CO_2 (conditions under which RuBP concentrations were presumably high), and obtained rates of up to 10 times higher than the steady-state rates of CO_2 assimilation! Lilley and Walker (1975) at Sheffield reached a similar conclusion after comparing the CO_2 responses of illuminated isolated chloroplasts with those obtained upon lysing chloroplasts in a medium containing saturated RuBP.

In our model of C_3 photosynthesis (Farquhar *et al.* 1980) the way we handled rate limitation by RuBP regeneration was probably the most important decision made in that context. Both ATP and NADPH were required for RuBP regeneration, and this fundamental need formed a connection with light in our model. From a mathematical perspective there were two options: (1) RuBP and CO_2 could always colimit the rate of carboxylation, and this we would express in a dou-

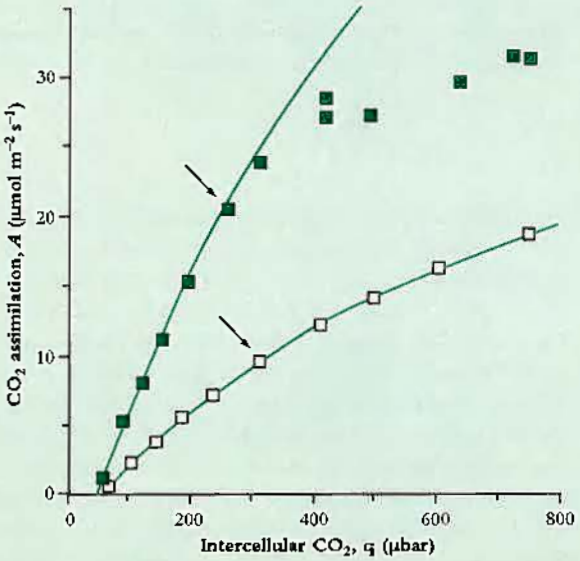


Figure 3 Transgenic tobacco with reduced amount of Rubisco shows no limitation by the rate of RuBP regeneration. CO_2 assimilation response curves in wild-type tobacco, ■, and in transgenic tobacco with reduced amount of Rubisco, □, were measured at a photon irradiance of 1000 μmol quanta m^{-2} s^{-1} and a leaf temperature of 25°C. Lines show Rubisco-limited rates of CO_2 assimilation (see legend to Figure 2) The reduction in Rubisco in transgenic tobacco was achieved with an antisense gene directed against the mRNA of the Rubisco small subunit (Hudson *et al.* 1992). Arrows indicate the points obtained at an external CO_2 concentration of 350 μbar

ble Michaelis Menten equation, or (2) carboxylation rate could be limited by either RuBP or else saturated and thus independent of RuBP. The *in vivo* kinetics of Rubisco snggest the second option.

Peisker (1974) and Farqnhar (1979) pointed out that Rubisco was unusnal in that it was present in the chloroplast at very high concentrations. Given such a low K_m(RuBP), this meant that the *in vivo* kinetics with respect to chloroplastic RuBP were those of a tight binding substrate. That is, the rate of Rubisco would depend linearly on RuBP concentration when chloroplastic RuBP concentration was below Rnbisco catalytic site concentration, and once RuBP exceeded Rubisco site concentratiou carboxylase would be RuBP satnrated. We also knew that irradiance affected CO_2 assimilation rate mainly at high intercellular CO_2. This supported option 2 (see Figure 2(a), (b)). Given these insights, the more complex link between chloroplastic electron transport rate and RuBP pools used by Farqnhar *et al.* (1980) was quickly simplified to a description of CO_2 assimilation that was limited by RuBP regeneration, and utilisation of ATP and NADPH for photosynthetic carbon reduction or oxygenation. RuBP regeneration was in turndriven by the electron transport rate, J (dependent on irradiance and its own maximal capacity), and stoichiometry of ATP or NADPH nse by the photosynthetic carbon reduction and oxygenation cycle. For example, when electron transport rate, J, was limiting considering ATP use, carboxylation rate could proceed at:

$$V_c = J/(4.5 + 10.5\Gamma\star/C) \quad (7)$$

Dashed lines in Figure 2 give modelled electron transport limited rates of CO_2 fixation according to:

$$A = \frac{(c_i - \Gamma\star)}{4.5c_i + 10.5\Gamma\star} \quad (8)$$

This simplified formnlation of C_3 photosynthesis (Equations 6 and 8) now provides a meaningful framework for analysis of leaf photosynthesis, and has focused our interpretation of CO_2 response curves on leaf biochemistry. For example, von Caemmerer and Farquhar (1981) related the initial slopes of CO_2 response curves to *in vitro* Rubisco activity, and the CO_2-saturated rates of A:c_i curves to *in vitro* measurements of electron transport rates. Such studies validate Equations 6 and 8, demonstrating that CO_2 response curves conld be used as a meaningful and non-invasive tool to quantify these biochemical components under a wide variety of conditions. Subsequent comparisons between wild-type tobacco and transgenic tobacco with a reduced amount of Rubisco have confirmed our concepts. When Rubisco alone is reduced in transgenic plants, RuBP regeneration capacity remains unchanged and no longer limits the rate of CO_2 assimilation at high CO_2. Rubisco then constitutes the sole limitation (Figure 3).

Colimitation

Both Rubisco and electron transport components are expensive in terms of leaf nitrogen. For example, Rubisco represents up to 25% of aleaf's protein nitrogen, with energy transduction components a further 25%. At a c_i where the transition from a Rubisco limitation to RuBP regeneration limitation occurs, both capacities are used efficiently and colimit net CO_2 assimilation. That is, assimilation can only be increased if both sets of component processes are increased. Where then should the balance lie if a plant is to use nitrogen-based resources to best effect? The transition obviously varies with irradiance and temperature so that an optimal balance will vary with habitat. However, snrprisingly little variation has beeu observed and plants appear unable to shift this point of balance. As an example, important in the context of rising atmospheric CO_2 concentrations, plants grown in a high CO_2 environment should manage with less Rubisco and thus put more nitrogen into the capacity of RuBP regeneration. Surprisingly, such adjustments have not been observed experimentally, but given prospects of global change, our need for understanding gains urgency.

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The plateau in Figure 1.6 at high irradiance is set by maximum Rubisco activity. With increasing CO_2 partial pressure, the rate of carboxylation increases along with a reduction in photorespiration. Note, however, that the transition from light-limited to Rubisco-limited CO_2 assimilation as irradiance increases is not a classic Blackman curve (i.e. with a sharp transition), and becomes progressively more gradual at higher CO_2 partial pressures. In part, this gentle transition reflects the fact that a leaf is a population of chloroplasts which have different photosynthetic properties depending on their position within that leaf. As discussed above, the profile of photosynthetic capacity per chloroplast changes less than the profile of light absorption per chloroplast (Figure 1.4). This results in an increase in CO_2 fixed per quanta absorbed with increasing depth. A transition from a light to a Rubisco limitation therefore occurs at progressively higher incident irradiances for each subsequent layer and results in a more gradual transition in the irradiance response curve of a leaf compared to that of a chloroplast.

Photosynthetic capacity of leaves varies widely according to light, water and nutrient availability and these differences in capacity usually reflect Rubisco content. Leaves in high light environments ('sun' leaves) have greater CO_2 assimilation capacities than those in shaded environments and this is reflected in the larger allocation of nitrogen-based resources to photosynthetic carbon reduction (PCR cycle; Section 1.1). Sun leaves have a high stomatal density, are thicker and have a higher ratio of Rubisco to chlorophyll in order to utilise the larger availability of photons (and hence ATP and NADPH). Shade leaves are larger and thinner, but have more chlorophyll per unit leaf dry weight than sun leaves. They can have a greater quantum yield per unit of carbon invested in leaves, but with a relatively greater allocation of nitrogen-based resources to photon capture, shade leaves achieve a lower maximum rate of assimilation.

Despite such differences in leaf anatomy and chloroplast composition, leaves sustain energy transduction and CO_2 fixation in an efficient and closely coordinated fashion. Processes responsible are discussed below (Section 1.2).

1.2 Chloroplasts and energy capture

In thermodynamic terms, O_2-generating photosynthesis in vascular plants is an improbable process! Improbable, because a weak oxidant (CO_2) must oxidise a weak reductant (H_2O), thereby producing a strong oxidant (O_2) and a strong reductant (carbohydrate). To achieve this 'uphill' reaction, a massive and continuous input of chemical energy is required. However, in nature, only radiant energy is available on that scale. How then can green plants achieve this conversion? Chloroplasts are responsible, and in what is unarguably the most significant process in our biosphere, photosynthetically active quanta are trapped and converted into chemically usable forms. This captured energy sustains plant growth and provides a renewable resource base for life on earth.

Thanks to the pioneering work of Calvin and Benson at Berkeley on $^{14}CO_2$ fixation products by *Chlorella* which began in the 1950s, biochemical aspects of photosynthetic carbon reduction (Calvin cycle) are now comprehensively

understood. Not so energy transduction, and while events surrounding photosynthetic electron flow are defined in some detail and are described here, biophysical processes within the water-splitting apparatus of chloroplasts, and indeed the manner in which photons are captured and their quantum energy harnessed for photolysis, remains something of an enigma and fall outside the scope of our present account.

1.2.1 Chloroplast structure and composition

Chloroplasts are easily recognised under a light microscope in leaf sections as distinctive green organelles suspended in the cytoplasm and usually appressed against cell walls. Chloroplasts are abundant in mesophyll tissue (commonly 200–300 in each palisade cell) and functional organelles can be isolated from homogenates of leaf tissue.

Chloroplasts are surrounded by a double membrane, or envelope just visible in transmission electron micrographs (Figure 1.7). This envelope encapsulates a soluble (gel-like) stroma which contains all the enzymes necessary for carbon fixation, many enzymes of nitrogen and sulphur metabolism and the chloroplast's own genetic machinery.

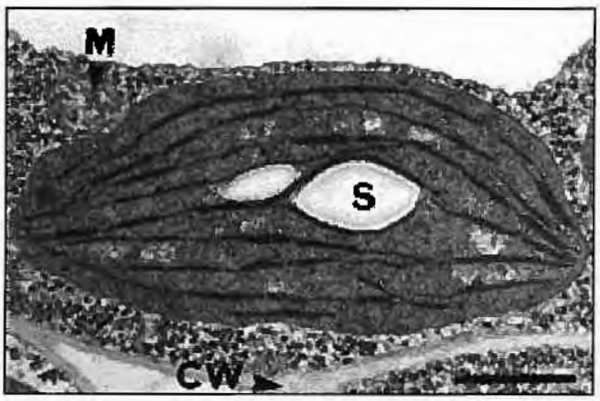

Figure 1.7 A mature and functional chloroplast in an immature leaf of bean (*Phaseolus vulgaris*) with an extensive network of photosynthetic membranes (thylakoids), parts of which are appressed into moderate granal stacks, and suspended in a gel-like matrix (stroma). The chloroplast contains a pair of starch grains (S) encapsulated in a double membrane (envelope) and suspended within a granular cytoplasmic matrix adjacent to a mitochondrion (M) and in close proximity to the cell wall (CW)
(Original transmission electron micrograph courtesy Stuart Craig and Celia Miller)

The inner membrane of a chloroplast envelope is an effective barrier between stroma and cytoplasm, and houses transporters for phosphate and metabolites (Section 2.2) as well as some of the enzymes for lipid synthesis. By comparison, the outer membrane of the chloroplast envelope is less complex and more permeable to both ions and metabolites.

Suspended within the stroma, and entirely separate from envelope membranes, is an elaborately folded system of photosynthetic membranes or 'thylakoids' (literally 'little sacs'). Embedded within these membranes are large populations of four basic complexes comprising two types of photosystem (with interlinked protein and pigment molecules), cytochrome *b/f* complexes (pivotal for photosynthetic electron transport) and ATP synthase complexes (responsible for proton egress from thylakoid lumen to stroma, and consequent ATP generation). Collectively, these complexes enable light harvesting and electron flow from H_2O molecules to $NADP^+$, thereby converting solar energy into chemically usable forms. This remarkable conversion, with such profound implications for life as we know it, starts with selective absorption of incoming light by chlorophylls and accessory pigments (certain carotenoids) that operate within both types of photosystem.

1.2.2 Chlorophyll absorption and photosynthetic action spectra

Chlorophylls are readily extracted from (soft) leaves into organic solvent and separated chromatographically into constituent types, most notably chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*). These two chemical variants of chlorophyll are universal constituents of wild vascular plants and express highly characteristic absorption spectra (Figure 1.8). Both chlorophylls show absorption maxima at wavelengths corresponding to blue and red, but chlorophyll assay in crude extracts which inevitably contain carotenoids as well is routinely based on absorption maxima in red light to avoid overlap with these accessory pigments that show strong absorption below 500 nm. Absorption maxima at 659 and 642 for Chl *a* and Chl *b* respectively would thus serve for assay in diethylether, but these peaks will shift slightly according to solvent system, and such shifts must be taken into account for precise measurement (see Porra *et al.* 1989 for details).

Chl *a* and Chl *b* differ with respect to both role and relative abundance in higher plants. Chl *a/b* ratios commonly range from ~~2.5~~ 3.3 to 4.2 3.0 in well-nourished sun-adapted species, but can be as low as ~~1.75~~ 2.2 or thereabouts in shade-adapted species grown at low light. Such variation is easily reconciled with contrasting functional roles for both Chl *a* and Chl *b*. Both forms of chlorophyll are involved in light harvesting, whereas special forms of only Chl *a* are linked into energy-processing centres of photosystems. In strong light, photons are abundant and warrant a substantial capacity for energy processing by leaves (hence the higher Chl *a/b* ratio). In weak light, optimisation of leaf function calls for greater investment of leaf resources in light harvesting rather than energy processing. As a result the relative abundance of Chl *b* will increase and the Chl *a/b* ratio will be lower compared with strong light. As a further subtlety, the two photosystems of higher plant chloroplasts (discussed later) also differ in their

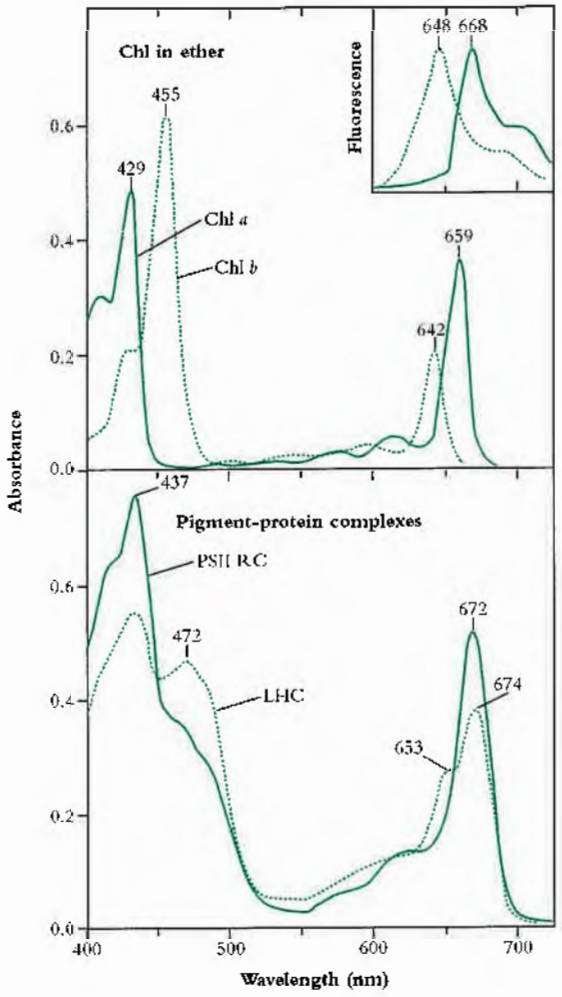

Figure 1.8 *Upper curves*: Diethylether solutions of chlorophyll *a* (Chl *a*, solid line) and chlorophyll *b* (Chl *b*, dotted line) show distinct absorption peaks in blue and in red regions of the visible spectrum (redrawn from Zscheile and Comar's (1941) original data). Fluorescence emission spectra (inset, redrawn from Lichtenthaler 1986) show peaks only in red, and at wavelengths characteristically longer than corresponding absorption peaks, namely 648 cf. 642 nm for Chl *b*, and 668 cf. 662 nm for Chl *a*. *Lower curves*: *In situ* absorption spectra (eluted from gel slices) for pigment–protein complexes corresponding to photosystem II reaction centre (PSII RC) and light-harvesting chlorophyll (*a*, *b*)–protein complexes (LHC). A secondary peak at 472 nm and a shoulder at 653 nm indicate contributions from Chl *b* to these broadened absorption spectra which have been normalised to 10 μM Chl solutions in a 1 cm path length cuvette
(Redrawn from Evans and Anderson 1987)

Chl *a*/*b* ratio, and provided Boardman and Anderson (1964) with the first clue that they had achieved a historic first in the physical separation of those two entities.

Carotenoids also participate in photosynthetic energy transduction. Photosystems have an absolute requirement for catalytic amounts of these accessory pigments, but their more substantive involvement is via dissipation of potentially harmful energy that would otherwise impact on delicate reaction centres when leaves experience excess photon irradiance (further details in Chapter 12). Carotenoids are thus regarded as 'accessory' to primary pigments (chlorophylls) and in molar terms are present in mature leaves at about one-third the abundance of Chl (*a* + *b*).

Obviously, chlorophyll in leaves is not in solution but exists in a gel-like state where all pigment molecules are linked to proteins, and absorption spectra differ accordingly (see Evans and Anderson 1987). In particular, light-harvesting Chl *a*, *b*–protein complexes (LHC in Figure 1.10, lower curves) develop a secondary absorption peak at 472 nm with a shoulder at 653 nm, while the Chl *a* of photosystem II reaction centres shows absorption peaks at 437 and 672 nm (compared with 429 and 659 nm for purified Chl *a* in solution; Figure 1.10, upper curves).

Subtle alterations in the molecular architecture of chlorophyll molecules according to the particular protein to which they bind in either light-harvesting or energy-processing centres are responsible for these shifts in absorption peaks, and for a general broadening of absorption spectra (compare lower and upper curves in Figure 1.8). Such effects are further accentuated within intact leaves by accessory pigments and greatly lengthened absorption pathways resulting in about 90% of visible wavelengths being absorbed (Figure 1.9). As a

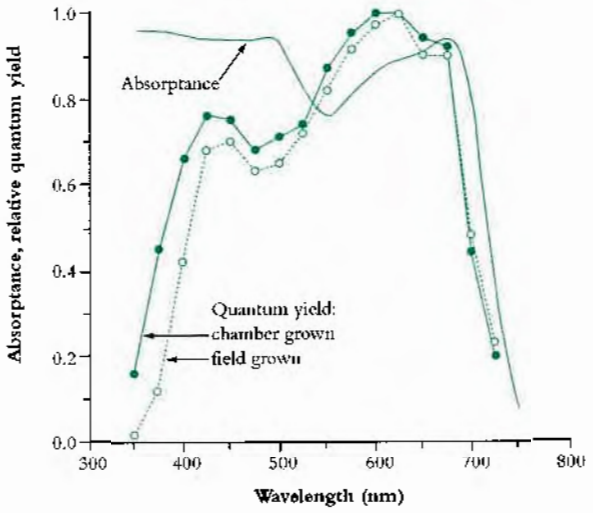

Figure 1.9 Leaves absorb visible light very effectively (>90% for all wavelengths combined; solid curve). Wavelengths corresponding to green light are absorbed less effectively (absorptance drops to *c*. 75%). Beyond 700 nm (infrared band) absorptance drops to near zero, and forestalls leaf heating from this source of energy. Quantum yield is referenced to values obtained in red light (600–625 nm), which is most effective in driving photosynthesis, requiring about 10 quanta per CO_2 assimilated (based on high-precision leaf gas exchange) compared with about 12 quanta at the blue peak (450 nm). Quantum yield thus shows a bimodal response to wavelength. Absorptance drops beyond 700 nm but quantum yield drops off even faster because PSII (responsible for O_2 generation) absorbs around 680 nm and cannot use quanta at longer wavelengths in this measuring system. UV wavelengths (below 400 nm) are capable of driving photosynthesis, but as a protective adaptation vascular plants accumulate a chemical 'sunscreen' in response to UV exposure. Field-grown plants are especially rich in these substances so that absorbed UV is dissipated harmlessly, lowering quantum yield compared with growth-chamber plants
(Redrawn from McCree 1972)

consequence, absorption maxima (Figure 1.8) and photosynthetic action maxima (Figure 1.9) become somewhat displaced.

Nevertheless, are these wavelengths all nsed to equal effect in photosynthesis? Clearly not, and major discrepancies between absorptance and quantnm yield occur at each end of the visible spectrum (Figure 1.9). In this bimodal action spectrnm, a minor peak around 450 nm and a major peak at 625 nm broadly match absorbance maxima of pigment–protein complexes. Although UV wavelengths are absorbed by leaves and would be capable of driving photosynthesis, such short wavelengths are damaging to biological systems and plants have adapted by developing a chemical sunscreen. Consequently, the quantum yield from these wavelengths drops off markedly below about 425 nm. Beyond 700 nm (infrared band) absorption drops to near zero, and forestalls leaf heating from this source of energy. However, quantum yield falls away even faster, and this 'red drop', though puzzling at first, led subsequently to a comprehensive model for photosynthetic energy transduction, outlined below.

1.2.3 Cooperative photosystems and a 'Z' scheme for electron flow

Prior to the advent of high-precision leaf gas exchange methods (as employed for Figure 1.9), O_2 evolution was taken as a measure of photosynthetic activity. Action spectra were measured on a number of plants and algae over the range of visible radiation. A crucial and consistent observation was that O2 evolution dropped off much faster in the long-wavelength red region (>690 nm) than did absorption. Put another way, more quanta were being absorbed at longer wavelengths than conld be used for photosynthesis. It seemed at these longer wavelengths as though a light absorher was being robbed of energy-processing capacity.

Anticipating that bimodal absorption implied a two-step process, and knowing that chlorophyll also absorbed photons at shorter wavelengths, Robert Emerson (working at Urbana in the mid-1950s) supplemented far-red light with shorter wavelength red irradiance and demonstrated that the relatively low photosynthetic rate in far-red light could be significantly increased. In fact the photosynthetic rate achieved with the two light qualities combined could be 30–40% higher than the sum of the rates in far-red or shorter red when measured separately (Emerson *et al.* 1957). This phenomenon became known as the 'Emerson Enhancement Effect' and contributed to a working hypothesis for photosynthetic energy conversion based upon two photochemical acts (proposed by Duysens *et al.* 1961), but additional lines of evidence were impacting on this outcome.

At about the same time as Emerson was establishing his enhancement effect, Myers and French observed 'sequential enhancement'; that is, a disproportionate increase in photosynthetic rate or efficiency when the two light qualities were separated in time. The upper limits of dark intervals between two flashes of different light quality were 6 s for far-red after green and and 1 min for green after far-red. Clearly, the 'product' of photochemical act 1 was stable for 1 min, that of act 2 for only 6 s. This discovery implied that chemical intermediates, rather than an altered physical state, were involved in a two-step cooperation (see Clayton 1980).

According to physical laws of photochemical equivalence, there should be a 1:1 yield in converting light energy to chemical energy by a perfect system. Quantum requirement for such events would be 1. However, in photosynthesis the absolute quantum requirement for O_2 evolution was clearly much greater than 1 and though contentious at first proved to be somewhere between 8 and 10. Such requirement implied a multistep process for energy conversion. Indeed, during the early 1940s a scientific controversy raged between Otto Warburg (Berlin) and Robert Emerson (Urbana) about the quantum requirement of photosynthesis in green algae and plants. Using manometry, a technique predating O_2 electrodes, Warburg related rates of O_2 evolution to absorbed quanta and claimed that 4 quanta were sufficient for the evolution of one molecule of O_2. By contrast, Emerson and many other scientists were reporting values of 6 to 12 quanta per molecule of O_2. The two sets of protagonists agreed to a collaborative effort, and by the late 1950s Emerson and co-workers resolved that 8–10 quanta were required.

How then could multiples of 2 quanta cooperate in the separation of one strong reducing and one strong oxidising equivalent? Everything started coming together when Hill and Bendall (1960) suggested a 'Z' scheme that was not only consistent with a requirement of 8–10 quanta and the 2 quanta cooperation principle, but also the operation of two sequential photochemical acts (Figure 1.10 is a greatly embellished version of their original model).

The original version of this 'Z' scheme was further validated by unequivocal evidence from Australia that the two (inferred) photosystems were indeed separate physical entities. Using sophisticated biochemical chloroplast purification and subfractionation methods, coupled with detergent solubilisation of membranes, Boardman and Anderson (1964) achieved the first physical separation of photosystem II (PSII) from photosystem I (PSI), thus confirming the separate identities of those complexes.

A source of electrons had long been recognised as basic to the operation of this 'Z' scheme, with H_2O molecules an obvious source, but were photosynthetic membranes capable of photolysis? Early experiments by Robin Hill and colleagues at Cambridge had established this capability. They used isolated thylakoid membrane preparations and showed that O_2 could be evolved in the absence of CO_2 as long as external electron acceptors were present (Hill reaction). Intact leaves or whole chloroplasts have no need for an artifical

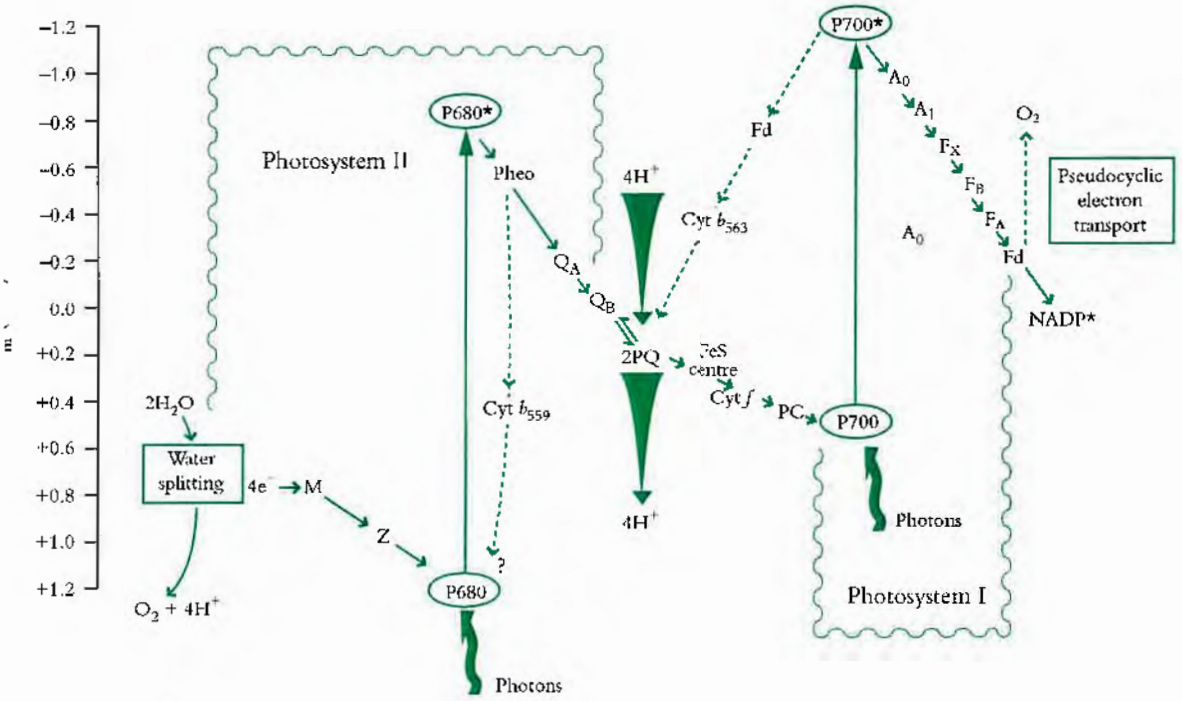

Figure 1.10 A highly diagrammatic zig-zag or 'Z' scheme of photosynthetic electron transport from water to NADP+ showing the sequence of electron/proton carriers and their association with either PSII or PSI. Linear electron flow is shown as solid lines; cyclic electron flow is indicated by dashed lines. All of these electron transport chains operate within thylakoid membranes with electron flow following a sequence dictated by redox potential (shown in volts on the ordinate). Cyclic electron flow in PSII diverts electrons from pheophytin to cytochrome b_{559} (and possibly back to $P680^+$). Cyclic electron transport around PSI moves electrons from ferredoxin through cytochrome b_{563} and plastoquinone (PQ), while pseudocyclic electron transport takes electrons from ferredoxin to O_2.

In linear flow, water molecules are split in PSII, liberating O_2 and providing a source of electrons. M is the manganese-containing cluster which oxidises water, Z is tyrosine-161 of the D1 protein which in turn represents the primary electron donor to $P680^+$ (a special pair of Chl *a* molecules with an absorption peak at 680 nm). Pheo is the primary electron acceptor pheophytin *a*, a chlorophyll molecule lacking Mg; Q_A is the first stable and permanently bound plastoquinone electron acceptor; Q_B is the second, temporarily bound, plastoquinone electron acceptor which actually leaves PSII in a reduced form (PQH_2). Further along, FeS = Rieske iron–sulphur centre; Cyt *f* = cytochrome *f*; PC = plastocyanin; P700 = reaction centre chlorophyll *a* of PSI; A_0, A_1, F_X, F_B, and F_A are electron acceptors of PSI; Fd = ferredoxin; Cyt b_{559} = cytochrome b_{559}; Cyt b_{563} = cytochrome b_6. Also shown as tapered arrows is H+ accumulation in the lumen associated with water and plastoquinol oxidations.

acceptor because electron flow is directed to $NADP^+$ and subsequent reduction of CO_2 (first demonstrated with intact chloroplasts; see Arnon 1984). The O_2-evolving function of photosynthesis was found to be associated with PSII in experiments with isolated thylakoids using external (artificial) electron donors and acceptors and specific electron transport inhibitors. As one outcome of those early Cambridge experiments, O_2 evolution is now measured routinely *in vitro* (and *in vivo* on leaves) with O_2 electrodes (Walker 1987).

Chloroplast structure and function is by now sufficiently well defined to consider photosynthetic electron flow in detail. Figure 1.10 applies equally well to vascular plants or to algae with oxygenic photosynthesis, where in either case two photosystems work cooperatively and sequentially in absorbing photons and converting their quantum energy into a flow of electrons. Paradoxically, convention has it that photosynthetic electron flow initiates in PSII and proceeds to PSI. PSII was so named because PSI had already been described in single-celled (prokaryotic) organisms and, owing to the rules of nomenclature, was accorded priority.

Both photosystems are large multi-subunit complexes, quite different structurally and functionally, and operating in series. In PSII, electrons are provided from a water-splitting apparatus via a manganese complex which undergoes oxidation from a valency state of +2 to +4. These oxidation states are made possible by $P680^+$ (a special form of Chl *a* with an absorption peak at 680 nm). $P680^+$ is a powerful oxidant generated by absorption of energy from a photon. P680 is referred to as a 'special pair' because it is a pair of Chl *a* molecules. Electrons from P680 pass to pheophytin (Pheo in Figure 1.10) and on to a bound quinone molecule, Q_A. From there a second transiently bound quinone Q_B receives two electrons in succession and requires protonation. The entire, fully reduced, quinone molecule leaves PSII and enters a plastoquinone pool (2PQ).

All of the electron transport cofactors of PSII and one β-carotene are bound to proteins D1 and D2. They in turn form a heterodimer, and together with cytochrome b_{559}, a 9 kDa phosphoprotein and a 22 kDa protein, form the PSII reaction centre. Attached to the reaction centre are core proteins

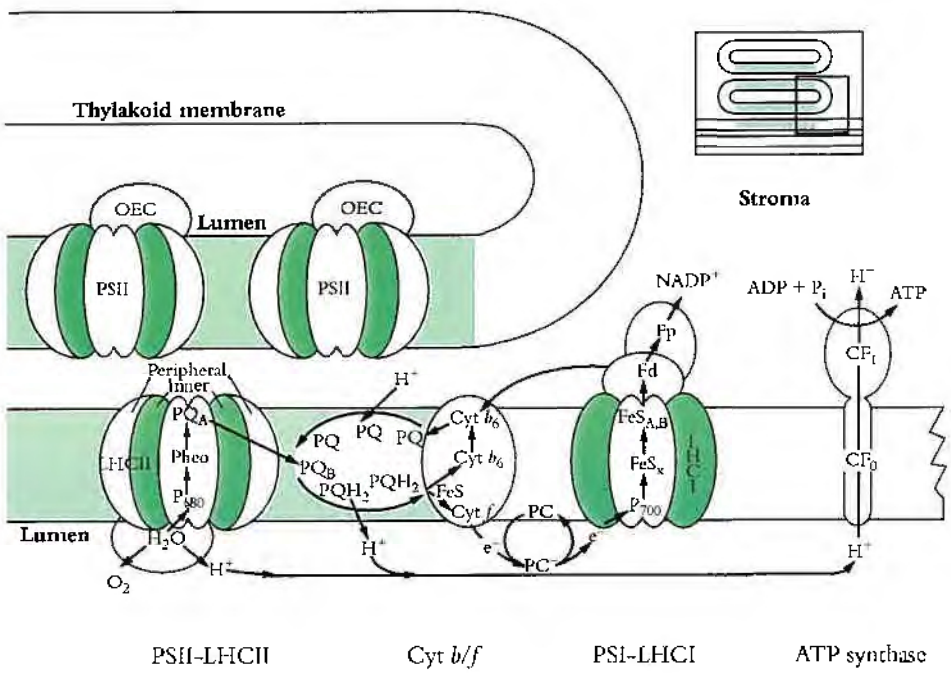

Figure 1.11 Light harvesting, photosynthetic electron transport from H_2O to $NADP^+$ and generation of ATP are achieved via four types of complexes which show a lateral heterogeneity within thylakoid membranes. A small part of a continuous network of interconnected thylakoids is shown here diagrammatically where PSI complexes and ATP synthase are restricted to non-appressed regions. Most PSII complexes, and the light-harvesting assemblages associated with PSII (LHCII) are held within appressed regions of this network. Cytochrome *b/f* complexes (Cyt *b/f*) are more generally located.

A chemiosmotic coupling mechanism is responsible for ATP synthesis. Protons are 'pumped' across the thylakoid membrane from outside (stroma) to inside (lumen) by a complex arrangement of electron carriers embedded within the membrane. A prodigious concentration of protons builds up within the lumen, partly from photolysis of water molecules (water-splitting apparatus on PSII) and partly from oxidation of plastoquinone (PQ) on the inner face of the membrane. This protonmotive force from inside (lumen) to outside (stroma) is used to generate ATP within the stroma via an ATP synthase complex that straddles the thylakoid membrane. OEC = oxygen-evolving complex; Pheo = pheophytin *a*

(Adapted from Anderson and Andersson 1988)

Based on

which carry 50 Chl *a* and carotenoid pigments. A heterogeneous light-harvesting chlorophyll–protein complex constitutes an outer antenna system and is composed of trimeric 28 kDa proteins which *in toto* bind 250–300 Chl (*a* + *b*) molecules per reaction centre. These 28 kDa proteins also bind carotenoids which provide one avenue for energy dissipation when photon capture exceeds subsequent energy utilisation (e.g. stressed plants in strong sunlight). Photons absorbed by the many pigments in LHCII are transferred as excitons by the LHCII and core pigments to reaction centres where they are finally trapped by P680. Such 'traps' are regarded as open if Q_A is oxidised and ready to receive an electron from P680. If, however, Q_A is still in its reduced state, that 'trap' will be closed, and excitons will then transfer to another reaction centre or be lost as heat or fluorescence. Traps have about one-billionth of a second (10^{-9} s) to accept an exciton before such energy is lost by either pathway.

In PSI, absorption of quantum energy from a photon causes oxidation of P700, the PSI reaction centre equivalent of P680. In contrast to PSII, where electrons are drawn from a water-splitting apparatus, P700 accepts electrons from PC (reduced form PC^- in Figure 1.11). Electrons then pass through three iron–sulphur (FeS) centres and out of PSI to ferredoxin (Fd). The reaction centre of PSI contains several proteins, but most of the electron transfer cofactors are bound to large heterodimeric proteins which in turn bind the inner Chl *a* antenna. The LHCI complex consists of possibly eight polypeptides of between 24 and 27 kDa which carry Chl *a* and Chl *b* plus carotenoids.

These two photosystems are juxtaposed across thylakoid membranes in such a way that linear electron transport is harnessed for charge separation, leading to a massive accumulation of H^+ ions within the lumen of illuminated thylakoids, which is then employed in ATP generation.

Combining concepts of photolysis and photosynthetic electron flow outlined earlier (Figure 1.10) and putting that conceptual framework into a thylakoid membrane system (Figure 1.11), a picture emerges where electrons generated from splitting H_2O molecules on the inner surface of PSII are transferred from plastoquinol (PQH_2) to the Rieske iron–

sulphur centre (Rieske FeS) of the cytochrome b_6/f complex (Cyt b_6/f) and further to cytochrome f (Cyt f). The pivotal importance of Cyt f in facilitatiug electron transport from PSII to PSI was demonstrated by Dnysens and colleagues (see Levine 1969), who showed that preferential energisation of PSII (light at <670 nm) caused rednction, whereas preferential energisation of PSI (light at >695 nm) caused oxidation. This elegant 'push–pull' experiment confirmed the cooperative and sequential nature of PSII and PSI, as well as indicating overall direction of photosynthetic electron flow.

Proteins which bind the Rieske FeS centre and Cyt f together with cytochrome b_{563} (Cyt b_6) form a large electron transfer complex. This complex (Figure 1.11) spans the membrane and is located between the two photosystems. Electrons are transferred to PC (forming PC^-), a copper-containing soluble protein extrinsic to the thylakoid membrane and located in the lumen. On tbe other side of the membrane, attached to the stromal side, is ferredoxin (Fd) which accepts electrons from PSI and passes them on to ferredoxin–NADP reductase, an enzyme, also extrinsic to thylakoids, and attached on the stromal side of the thylakoid membrane. This enzyme accomplishes the final electron transfer in an overall linear chain and reduced NADP is then protonated.

While linear electron transport from water to $NADP^+$ is the main and most important path, electrons can also be transferred to O_2 in a so-called pseudocyclic or Mehler reaction (Figure 1.10). This pathway probably operates *in vivo* as a sink for electrons when synthetic events call for more ATP than NADPH. Electrons can also be cycled around both PSII and PSI, again producing ATP with no accompanying NADPH. Cyclic electron flow around PSII may have a completely different role and may be related to the downregulation of this photosystem during photoinhibition (Chapter 12).

According to this multistage scheme, electrons are transferred from donor (reductant) to acceptor (oxidant). The direction of that transfer depends upon a difference in oxidation–reduction potential between a given donor and a given acceptor (as indicated on the ordinate in Figure 1.10). A more positive potential implies stronger oxidative power (i.e. capacity to accept electrons); a more negative potential implies stronger reducing power (i.e. capacity to donate electrons). P680* thus has a strong capacity to donate electrons (a strong reductant); P700* has an even stronger capacity to donate electrons (an even stronger reductant).

Molecules which accept electrons are immediately protonated. In aqueous systems, such as chloroplasts *in vivo*, hydrogen ions (H^+) are ubiquitous, and these ions combine with electron acceptors to generate hydrogen atoms (i.e. H^+ ion + electron → H atom). In Figure 1.10, some events involve electron transfer, while others include transfer of hydrogen atoms. As a simplifying convention, all snch events are referred to as electron transfers. Ironically, the end result of all these reactions is a net transfer of hydrogen atoms!

1.2.4 Photophosphorylation and ATP synthesis

During photosynthetic electron transfer from water to $NADP^+$, energy captured in two photoacts is stored as an electrochemical potential gradient of protons. First, such reduction of Q_B requires protonation with protons drawn from the stromal side of the membrane. Re-oxidation (and deprotonation) occurs towards the thylakoid lumen. In addition, protons are lost from the stromal side via protonation of reduced NADP and they are also generated in the lumen during photolysis. A massive ΔpH, of approximately 3–4 pH units, equivalent to an H^+ ion concentration difference of three to four orders of magnitude, develops across the thylakoid membrane. This immense gradient drives ATP synthesis (catalysed by ATP synthase) within a large energy transducing complex embedded in the thylakoid membrane (Figure 1.11).

ATP synthesis in chloroplasts (photophosphorylation) proceeds according to a mechanism that is basically similar to that in mitochondria. Chemiosmotic coupling (Mitchell 1961) which links the movement of protons down an electrochemical potential gradient to ATP synthesis via an ATP synthase applies in both organelles. However, the orientation of ATP synthase is opposite. In chloroplasts protons accumulate in thylakoid lumen and pass outwards through the ATP synthase into the stroma. In mitochondria, protons accumulate within the intermembrane space and move inwards, generating ATP and oxidising NADH within the matrix of these organelles (Fig 2.24).

In chloroplasts, ATP synthase is called the CF_oCF_1 complex. The CF_o unit is a hydrophobic transmembrane multiprotein complex which contains a water-filled proton conducting channel. The CF_1 unit is a hydrophilic peripheral membrane protein complex that protrudes into the stroma. It contains a reversible ATPase and a gate which controls proton movement between CF_o and CF_1. Entire CF_oCF_1 complexes are restricted to non-appressed portions of thylakoid membranes due to their bulky CF_1 unit.

Direct evidence for ATP synthesis due to a transthylakoid pH gradient can be adduced as follows. When chloroplasts are stored in darkness in a pH 4.0 succinic acid buffer (i.e. a proton-rich medinm), thylakoid lumen equilibrate to this pH. If the chloroplasts, still in the dark, are rapidly transferred to a pH 8.0 buffer containing ADP and Pi, ATP synthesis then occurs. This outcome confirms a central role for the proton concentration difference between thylakoid lumen and stroma for ATP synthesis *in vitro*; but does such a process operate on that scale *in vivo*?

Mordhay Avron, based in Israel, answered this question in part during the early 1970s via a most elegant approach (Rottenberg *et al.* 1972). Working with thylakoid preparations,

Avron and colleagues established that neutral amines were free to exchange between bathing medium and thylakoid lumen, but once protonated in illuminated preparations they became trapped inside. By titrating the loss of such amines from the external medium when preparations where shifted from dark to light, they were able to infer the amount retained inside. Knowing that the accumulation of amine depended upon H^+ ion concentration in that lumen space, the difference in H^+ ion concentration and hence ΔpH across the membrane were established.

At saturating light, chloroplasts generate a proton gradient of approximately 3.5 pH units across their thylakoid membranes. Protons for this gradient are derived from the oxidation of water molecules occurring towards the inner surface of PSII and from transport of four electrons through the Cyt *b/f* complex, combined with cotranslocation of eight protons from the stroma into the thylakoid space for each pair of water molecules oxidised. Electrical neutrality is maintained by the passage of Mg^{2+} and Cl^- across the membrane, and as a consequence there is only a very small electrical gradient across the thylakoid membrane. The electrochemical potential gradient that yields energy is thus due almost entirely to the concentration of intrathylakoid H^+ ions.

For every three protons translocated via ATP synthase, one ATP is synthesised. Linear electron transport therefore generates about four molecules of ATP per O_2 evolved. Thus eight photons are absorbed for every four ATP molecules generated or for each O_2 generated. Cyclic electron transport is slightly more efficient in producing ATP and generates about four ATP per six photons absorbed. However, linear electron transport also generates NADPH, which is the equivalent, in energy terms, to six ATP per O_2 released.

As implied in Figure 1.11, the four thylakoid complexes, PSII, PSI, Cyt *b/f* and ATP synthase, are not evenly distributed in plant thylakoid membranes but show a lateral heterogeneity. This distribution is responsible for the highly characteristic structural organisation of the continuous thylakoid membrane into two regions, one consisting of closely appressed membranes or granal stacks, the other of non-appressed stroma lamellae where outside surfaces of thylakoid membranes are in direct contact with the stroma. This structural organisation is shown on a modest scale in Figure 1.9 but extreme examples are evident in chloroplasts of shade-adapted species grown in low light (Chapter 13). Under such conditions, membrane regions with clusters of PSII complexes and Cyt *b/f* complexes become appressed into classical granal stacks. Cyt *b/f* complexes are present inside these granal stacks as well as in stroma lamellae, but PSI and ATP synthase are absent from granal stacks. Linear electron transport occurs in granal stacks from PSII in appressed domains to PSI in granal margins. Nevertheless, shade plants have only a low rate of linear electron transport because they have fewer PSII complexes compared to PSI, a consequence of investing more chlorophyll in each PSII to enhance light harvesting (see Anderson (1986) and Chapter 13 for more detail).

1.2.5 Chlorophyll fluorescence

A dilute solution of leaf chlorophyll in organic solvent appears green when viewed with light transmitted from a white source. Wavelengths corresponding to bands of blue and red have been strongly absorbed (Figure 1.8), whereas mid-range wavelengths corresponding to green light are only weakly absorbed, hence the predominance of those wavelengths in transmitted and reflected light. However, viewed laterally via re-emitted energy, the solution will appear deep red, and that same colour will persist regardless of source light quality. Fluorescence spectra are invariate, and the same spectrum will be obtained (e.g. Figure 1.8 inset) regardless of which wavelengths are used for excitation. This characteristic emission is especially valuable in establishing source pigments responsible for given emission spectra, and for studying changes in their photochemical status during energy transduction.

Fluorescence emission spectra (Figure 1.8 inset) are always displaced towards longer wavelengths compared with corresponding absorption spectra (Stoke's shift). As quantum physics explains, photons intercepted by the chromophore of a chlorophyll molecule cause an instantaneous rearrangement of certain electrons, lifting that pigment molecule from a ground state to an excited state which has a lifetime of *c.* 10^{-9} s. Some of this excitation energy is subsequently converted to vibrational energy which is acquired much more 'slowly' by much heavier nuclei. A non-equilibrium state is induced, and molecules so affected begin to vibrate rather like a spring with characteristic periodicity, leading in turn to energy dissipation as heat plus remission of less energetic photons of longer wavelength.

Apart from their role in photon capture and transfer of excitation energy, photosystems function as energy converters because they are able to seize photon energy rather than lose as much as 30% of it through fluorescence as do chlorophylls in solution. Moreover, they can use the trapped energy to lift an electron to a higher energy level from where it can commence a 'downhill' flow via a series of electron carriers as summarised in Figure 1.10.

Protein structure confers very strict order on bound chlorophylls. X-ray crystallographic resolution of the bacterial reaction centre has given us a picture of the beautiful asymmetry of pigment and cofactor arrangements in these reaction centres, and electron diffraction has shown us how chlorophylls are arranged with proteins that form the main light-harvesting complexes of PSII. This structural constraint confers precise distance and orientation relationships between the various chlorophylls, as well as between chlorophylls and

carotenoids, and between chlorophylls and cofactors enabling the photosystems to become such effective photochemical devices. It also means that only 2–5% of all the energy that is absorbed by a photosystem is lost as fluorescence.

If leaf tissue is held at liquid nitrogen temperature (77 K), photosynthetic electron flow ceases and chlorophyll fluorescence does increase, including some emission from PSI. Induction kinetics of chlorophyll fluorescence at 77 K have been used to probe primary events in energy transduction, and especially the functional state of photosystems. Present discussion is restricted to room temperature fluorescence where even the small amount of fluorescence from PSII is diagnostic of changes in functional state. This is because chlorophyll fluorescence is not emitted simply as a burst of red light following excitation, but in an ordered fashion that varies widely in flux during continuous illumination. These transient events (Figure 1.12) are referred to collectively as fluorescence induction kinetics, fluorescence transients, or simply a Kautsky curve in honour of its discoverer Hans Kautsky (Kautsky and Franck 1943).

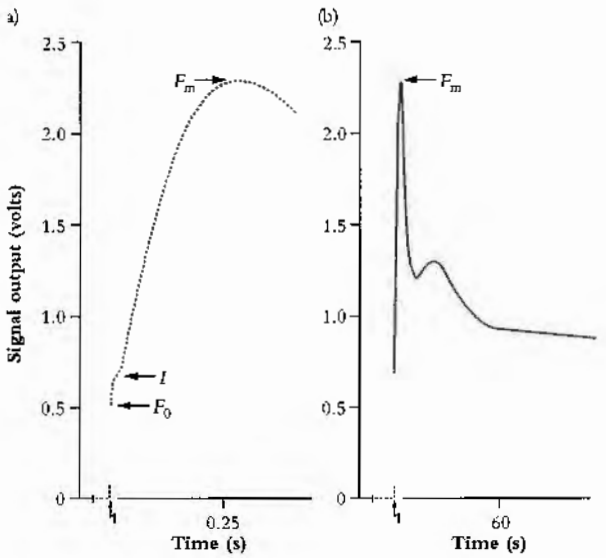

Figure 1.12 A representative chart recorder trace of induction kinetics for Chl *a* fluorescence at room temperature from a mature bean leaf (*Phaseolus vulgaris*). The leaf was held in darkness for 17 min prior to excitation (zig-zag arrow) at a photon irradiance of 85 μmol quanta m^{-2} s^{-1}. The overall Kautsky curve is given in (b), and an expanded version of the first 400 ms is shown in (a). See text for explanation of symbols and interpretation of variation in strength for these 'rich but ambiguous signals'!
(Redrawn from Nortish *et al.* 1983)

At room temperature and under steady-state conditions, *in vivo* Chl *a* fluorescence from an immature (greening) bean leaf shows a characteristic emission spectrum with a distinct peak around 690 nm and a shoulder near 735 nm. As leaf chlorophyll concentration increases, the intensity of fluorescence at 690 nm diminishes compared with emission at 735 nm due to reabsorption of shorter wavelengths by the extra chlorophyll molecules. Fully developed bean leaves thus show a new maximum between 730 and 740 nm. Room temperature fluorometers rely on this secondary peak.

(a) Fluorescence induction kinetics

Strength of emission under steady-state conditions varies according to the fate of photon energy captured by LHCII, and the degree to which energy derived from photosynthetic electron flow is gainfully employed. However, strength of emission fluctuates widely during induction (Figure 1.12) and these rather perplexing dynamics are an outcome of some initial seesawing between photon capture and subsequent electron flow. Taking Figure 1.10 for reference, complexities of a fluorescence transient (Figure 1.12) can be explained as follows. At the instant of excitation (zig-zag arrow), signal strength jumps to a point called F_o which represents energy derived largely from chlorophyll molecules in the distal antennae of the LHCII complex which fail to transfer their excitation energy to another chlorophyll molecule, but lose it immediately as fluorescence. F_o thus varies according to the effectiveness of coupling between antennae chlorophyll and reaction centre chlorophyll, and will increase due to high-temperature stress or photodamage. Mn-deficient leaves show a dramatic increase in F_o due to loss of functional continuity between photon harvesting and energy processing centres of PSII (discussed further in Chapter 17).

Returning to Figure 1.12, the slower rise subsequent to F_o is called I, and is followed by a further rise to F_m. These stages reflect a surge of electrons which fill successive pools of various electron acceptors of PSII. Significantly, F_m is best expressed in leaves that have been held in darkness for at least 10–15 min. During this dark pretreatment, electrons are drawn from Q_A, leaving this pool in an oxidised state and ready to accept electrons from PSII. An alternative strategy is to irradiate leaves with far-red light to energise PSI preferentially, and so draw electrons from PSII via the Rieske FeS centre. The sharp peak (F_m) is due to a temporary restriction on electron flow downstream from PSII. This constraint results in maximum fluorescence out of PSII at about 500 ms after excitation in Figure 1.12. That peak will occur earlier where leaves contain more PSII relative to electron carriers, or in DCMU-treated leaves.

Photochemistry and electron transport activity always quench fluorescence to a major extent unless electron flow out of PSII is blocked. Such blockage can be achieved with the herbicide 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU) which binds specifically to the D1 protein of PSII and blocks electron flow to Q_B. DCMU is a very effective herbicide because it inhibits photosynthesis completely. As a consequence, signal rise to F_m is virtually instantaneous, and fluorescence emission stays high.

Variation in strength of a fluorescence signal from F_o to F_m is also called variable fluorescence (F_v) because scale and kinetics of this rise are significantly influenced by all manner of environmental conditions. F_o plus F_v constitute the maximal fluorescence (F_m) a leaf can express within a given mea-

suring system. The F_v/F_m ratio, measured after dark treatment, therefore reflects the proportion of efficiently working PSII units among the total PSII population. Hence it is a measure of the photochemical efficiency of a leaf, and correlates well with other measures of photosynthetic effectiveness (discussed further in Chapter 12).

(b) Fluorescence relaxation kinetics

Excellent fluorometers for use in laboratory and field such as the Plant Efficiency Analyser (Hansatech, King's Lynn, UK) make accurate measurements of all the indices of the Kautsky curve and yield rapid information about photochemical capacity and response to environmental stress.

Even more sophisticated is the Pulse Amplitude Modulated (PAM) fluorometer (Walz, Effeltrich, Germany) which employs a number of fluorescence- and/or photosynthesis-activating light beams and probes fluorescence status and quenching properties. In contrast to induction kinetics generated by conventional fluorometers (e.g. Figure 1.12) where a given source of weak light (commonly a red light-emitting diode producing only 50–100 μmol quanta m^{-2} s^{-1}) is used for both chlorophyll excitation and as a source of light for photosynthetic reactions, a PAM fluorometer applies pulses of saturating light for chlorophyll excitation on top of an actinic beam which sustains photosynthesis. A combination of optical filters plus sophisticated electronics ensures that detection of fluorescence emission is locked exclusively onto the modulated signal. In this way, most of the continuous background fluorescence and reflected long-wavelength light is disregarded. The functional condition of PSII in actively photosynthesising leaf tissue is thus amenable to analysis. This instrument also reveals the relative contributions to total fluorescence quenching by photochemical and non-photochemical processes and will help assess any sustained loss of quantum efficiency in PSII. Photosynthetic electron transport rates can be calculated concurrently.

Photochemical quenching (q_p) varies according to the oxidation state of electron acceptors on the donor side of PSII. When Q_A is oxidised (e.g. subsequent to dark pretreatment), quenching is maximised. Equally, q_p can be totally eliminated by a saturating pulse of excitation light that reduces Q_A, so that fluorescence yield will be maximised, as in a PAM fluorometer. Concurrently, a strong beam of actinic light drives photosynthesis (maintaining linear electron flow) and sustaining a pH gradient across thylakoid membranes for ATP synthesis. Those events are a prelude to energy utilisation and contribute to non-photochemical quenching (q_n). This q_n component can be inferred from a combination of induction plus relaxation kinetics.

In Figure 1.13, a previously darkened radish leaf (traps now open and Q_A oxidised) initially receives weak modulated light (<1 μmol quanta m^{-2} s^{-1}) that is insufficient to close traps but sufficient to establish a base line for constant yield fluorescence (F_o). This value will be used in subsequent calculations of fluorescence indices. The leaf is then pulsed with a brief

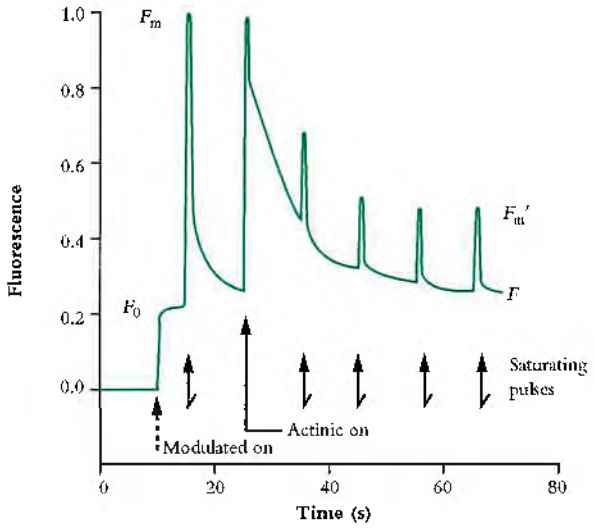

Figure 1.13 Induction and relaxation kinetics of *in vivo* Chl *a* fluorescence from a well-nourished radish leaf (*Raphanus sativus*) supplied with a photon irradiance of actinic light at 500 μmol quanta m^{-2} s^{-1} and subjected to a saturating pulse of 9000 μmol quanta m^{-2} s^{-1} for 0.8 s every 10 s. Output signal was normalised to 1.0 around the value for F_m following 30 min dark pretreatment. Modulated light photon irradiance was <1 μmol quanta m^{-2} s^{-1}). See text for definition of symbols and interpretation of kinetics (Original (unpublished) data from John Evans generated on a PAM fluorometer (Heinz Walz GmbH, Germany))

(0.8 s) saturating flash (9000 μmol quanta m^{-2} s^{-1}) to measure F_m. Pulses follow at 10 s intervals to measure F_m'. Actinic light (500 mmol quanta m^{-2} s^{-1}) starts with the second pulse and ΔpH starts to build up in response to photosynthetic electron flow. Photosynthetic energy transduction comes to equilibrium with these conditions after a minute or so, and fluorescence indices q_n and q_p can then be calculated as follows:

$$q_n = (F_m - F_m') / (F_m - F_o), \text{ and}$$
$$q_p = (F_m' - F) / (F_m' - F_o) \qquad (1.1)$$

Under these steady-state conditions, saturating pulses of excitation energy are being used to probe the functional state of PSII, and by eliminating q_p the quantum efficiency of light-energy conversion by PSII (Φ_{PSII}) can be inferred:

$$\Phi_{PSII} = (F_m' - F) / F_m' \qquad (1.2)$$

If overall quantum efficiency for O_2 evolution is taken as 10 (discussed earlier), then the rate of O_2 evolution by this radish leaf will be:

$$\Phi_{PSII} \times \text{photon irradiance}/10 \text{ (μmol } O_2 \text{ } m^{-2} \text{ } s^{-1}) \qquad (1.3)$$

In summary, chlorophyll fluorescence at ambient temperature comes mainly from PSII. This photosystem helps to control overall quantum efficiency of electron flow and its functionality changes according to environmental and internal controls. In response to establishment of a ΔpH across

thylakoid membranes, and particularly when irradiance exceeds saturation levels, some PSII units become down-regulated, that is, they change from very efficient photochemical energy converters into very effective energy wasters or dissipators (Chapter 12). Large amounts of the carotenoid pigment zeaxanthin ensure this harmless dissipation of energy as heat (other mechanisms may also contribute). PSII also responds to feedback from carbon metabolism and other energy-consuming reactions in chloroplasts, and while variation in pool size of phosphorylated intermediates has been implicated, these mechanisms are not yet understood.

1.3 Conclusion

Chloroplasts are sites of solar energy absorption and subsequent transduction into chemically usable forms. Splitting water molecules and developing a proton motive force of sufficient magnitude to drive ATP synthesis are energy-intensive processes. Consequently, photosynthetic organisms evolved with dual photosystems that work cooperatively and sequentially to extract sufficient quantum energy from parcels of absorbed photons to generate a sufficiently strong electrochemical potential gradient to synthesise the relatively stable, high-energy compounds ATP and NADPH. Such metabolic energy sustains cycles of photosynthetic carbon reduction (PCR) where CO_2 is initially assimilated by one of three photosynthetic pathways, namely C_3, C_4 or CAM, but eventually fixed via a PCR cycle within the stromal compartment of chloroplasts (considered further in Section 2.1).

Thermodynamically, the net outcome of photosynthetic energy transduction must be viewed as long-term storage of energy in the form of a *product pair*, namely free oxygen and reduced carbon (organic matter), rather than as separate molecules. Plants themselves or indeed any heterotrophic organisms subsequently retrieve such energy via metabolic 'combustion' of the organic matter where enzyme-catalysed reactions bring this pair of products together again. These sets of events are outlined in Section 2.4.

Further reading

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